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The Effect of Zinc and Lysine Supplementation on Infection Rate and CD4 Count In Elderly

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Abstract

Elderly people tend to have higher susceptibility of infections because immune dysfunction, especially cell-mediated immune system which is related to zinc deficiency. Lysine can support zinc role to boost up the cell-mediated immune system which can be determined by CD4 count. The objective of this study is to determine the effect of zinc and lysine supplementation on infection rate and CD4 count in elderly.

A randomized, double-blind, placebo-controlled trial was conducted in a senior center in Surabaya using 24 healthy elderly subjects of both sexes aged 62 to 90 years. They were divided into two experimental groups and one control group. They were given zinc 20 mg per day; or zinc 20 mg and lysine 500 mg per day; or placebo for 2 months. Infection rate during supplementation period was documented. Albumin level, serum zinc level and CD4 count were measured before and after supplementation. The data was analyzed using one way Anova and paired T-test with $p < 0.05$.

Compared to control group, infection rate was lower in zinc group and zinc + lysine group ($p < 0.065$). Zinc + lysine supplementation increased serum zinc level significantly ($p < 0,012$) and had better effect compared to zinc supplementation alone. Zinc + lysine supplementation also increased CD4 count ($p < 0,784$) and had better effect compared to zinc supplementation alone. Zinc + lysine supplementation did not increase albumin level which was already in the normal level. Zinc + lysine supplementation can reduce infection rate in elderly by increasing zinc level and CD4 count.

Keywords: Zinc; Lysine; CD4; Elderly

Introduction

Generally, life expectancy of elderly (seniors) in Indonesia is increasing due to the success of health sector development in Indonesia. Now Indonesia has entered a period of aging structured population because the number of people aged 60 years and over in Indonesia in 2010 reached 18.04 million or 7.59% of the population [1]. In 2025 the number of elderly is expected 13.2% and being 25.5% of the total population in 2050 [2].

Except degenerative diseases, the health problem faced by the elderly is an infectious disease that is usually caused by a decrease in the immune system, particularly cell-based immunity. Generally, in the elderly, the cell-based immune system (cell-mediated immunity) showed more decrease compared to humoral immunity. The capacity of T lymphocytes in the peripheral blood of elderly to multiply after a stimulus is lower compared to younger age groups. In elderly found a decrease of lymphocytes in the peripheral blood with age. In healthy elderly, the reduction only about 10-15% of normal lymphocytes amount [3].

Infections that are common in the elderly are respiratory infection, including pneumonia, influenza and urinary tract infections. Pneumonia often cause of death in the elderly. When influenza attacking the elderly who are already suffering from other chronic diseases such as kidney failure or heart failure can also cause death. While urinary tract infection is one of the most common cause of bacteremia or sepsis in elderly [4]. Based on research conducted in the Kariadi Hospital in 1991 to 1994, the most infectious disease in the elderly is bronchopneumonia, urinary tract infection, sepsis and gastroenteritis. The highest death rate in the elderly due to infection caused by sepsis and bronchopneumonia [5].

The decline of immune system in the elderly caused by the aging process and macronutrient deficiency such as energy and protein (which includes lysine amino acid) and micronutrients, one of them is zinc [3,6]. Zinc deficiency is common in the elderly and usually accompanied by atrophy of thymus gland as a result of aging process, which is characterized by a decrease in the thymulin hormone and IL-2 production (interleukin 2). Decrease of thymulin hormone which produced by thymus gland is characterized by a decrease in thymulin activity serum (thymulin activity). Thymulin is required for differentiation and maturation process of T helper cells (Th1). Thymulin are tightly bound to the T cell receptor, requires zinc as a co-factor for its biological activity. Except has a role in adaptive immunity such as T and B lymphocytes, zinc also serves to keep the innate immune cells such as neutrophils, monocytes, macrophages, natural killer cells can function normally [7-9]. Zinc deficiency also results in impaired balance of Th1/Th2, resulting in decreased production of Th1 cytokines, especially IFN- and IL-2 that are required for optimal activity of macrophage phagocytosis against parasites, viruses and bacteria. It also causes infections occur more easily [10].

Lysine, one of the essential amino acids, has an important role in the immune system, including preventing recurrence of herpes simplex virus [11], reducing morbidity as a result of diarrhea and bronchopneumonia [12]. Lysine supplementation can increase levels of CD3, C3, Ig G, Ig A, Ig M and CD4 [13,14]. In experiments using

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mice, lysine can directly increase the number of T lymphocytes by stimulating vagal afferent nerve system to the hypothalamus along with arginine (non-essential amino acids which derived from dietary sources of protein that similar to lysine). Then the hypothalamus stimulates vagal afferent and sympathetic nerve system to thymus gland, spleen and lymph nodes. The thymus gland after stimulated by the sympathetic and vagal efferent nerves will increase the release of T lymphocytes, including T helper cells, so CD4 counts will increase [15,16]. Lysine can also increase absorption of zinc. In other experiments in mice, lysine can increase zinc absorption in small intestine by forming a bond (ligand) with lysine, resulting in increased serum zinc levels [17]. In addition, lysine supplementation increased transferrin and pre-albumin serum, which reflects the improvement of protein status [13]. Transferrin serum, pre-albumin and albumin are required for the transport of zinc in the blood [18].

Aside from zinc and protein deficiency, other factors that facilitate occurrence of infections in elderly is low number of CD4 which T helper cell marker. CD4 used to control immune response through cytokines. When there is an infection, CD4 determine actions to be performed by other immune cells such as activated macrophages and induces proliferation of B cells and T cells. When thymulin activity serum decreased, Th1 will decrease and new CD4 cells will decrease too [3,8]. CD4 count is lower when there is an infection in the elderly [19]. Zinc deficiency also causes apoptosis of timosit cells in thymus gland, causing involution of thymus gland. At in vitro experiments, the provision of zinc can rescue cells from apoptosis [20].

Data infections in nursing homes in Indonesia still difficult to obtain, but it is estimated that respiratory tract infections, gastroenteritis and urinary tract infection are infectious diseases that often affects elderly in nursing homes in Indonesia. Several studies have shown zinc supplementation can reduce the frequency of occurrence and duration of respiratory infections and diarrhea [10,21]. While lysine supplementation which is one of the essential amino acids also serve to increase body's immune. Lysine supplementation can also reduce morbidity cause diarrhea and bronchopneumonia [12]. Hopefully, with zinc and lysine supplementation together have a greater effect to increase lymphocyte subsets (CD4), expected more elderly people rarely get infections, especially respiratory infections.

The purpose of this study is to determine the effect of zinc and lysine to increase the body's immune system (CD4) and infections in the elderly. The results of this study are expected to be able to add information about benefits of zinc and lysine to the elderly immunity, and can be used as inputs in implementation of programs relating to the elderly in order to realize the vision of "Lanjut Usia Indonesia Sejahtera 2020".

Methods

Study design and population

This experimental study using randomized pre-test post-test control group design. Minimum number of samples for the study were β4 samples with α = 0.05 and = 0.β. Treatment provision performed with double-blind on 27 people, consisting of 11 men and 16 women who live in a nursing home in Surabaya. They were divided into 3 groups. The first group was control group, the second group is treatment group and the third group also treatment group. In end of the research, the number of respondents is 24 people because there are 3 respondents who dropped out due to leave nursing home (1 person), diagnosed with advanced cancer (1 person) and 1 person resigned because did not want to continue taking supplements.

Informed consent and ethical clearance

The inclusion criteria of this study are 1) the elderly who live in a nursing home in S u r a b a y a , 2) at least 60 years old or more, 3) not in a state of chronic infections, cancer or organ failure, and 4) willing to participate in the study by signing the informed consent. This study has also been approved by ethical committee Public Health Faculty of Airlangga University.

Supplement

There are three kinds of supplements. Placebo syrup, 20 mg of zinc syrup (zinc sulfate) and mixture of 20 mg zinc syrup (zinc sulfate) and lysine 500 mg. Supplementation given 10 ml after breakfast for 2 months. All three kind of syrup made by PT Coronet Crown, Sidoarjo.

Data collection

Variables measured in this study were serum zinc levels, serum albumin levels and CD4 counts before (pre-test) and after supplementation (post-test) and the occurrences of infection during 2 months of supplementation.

Biochemistry assessment

Blood sampling as much as 9 ml and placed in vacutainer with EDTA anticoagulant. CD4 count measurements using flow cytometry with FACSCount BD tool. Measurement of serum albumin level is done by using Bromocresol Green (BCG) method. Both examinations are performed in Laboratory Prodia Surabaya that have ISO 9001 standard. While the method used to measure serum zinc levels was Atomic Absorption Spectrophotometry (AAS) and worked at Balai Besar Laboratorium Kesehatan Surabaya, which also has the ISO 9001 standard.

Statistical analysis

The hypothesis of this study is there is effect of zinc and lysine supplementation on incidence of infection and CD4 counts in the elderly. The hypothesis statistically analyzed using one-way ANOVA test. When data distribution is normal, it was tested with the Kolmogorov-Smirnov test and variance data homogeneous with Levene test. When the data distribution is not normal or variance data is not homogeneous, then the data will be tested with Kruskall Wallis test.

Result

Characteristic of respondents

From 24 elderly respondents who involved in the study until the end, 10 people are male and 14 female. Once mapped, the control group and zinc group had 3 male respondents (37.5%) and 5 female respondents (62.5%), whereas zinc + lysine groups have 4 male respondents (50%) and 4 female respondents (50%). Chi-square test was found that the differences proportion of elderly gender in three groups was not significant (p = 1.000).

Characteristics of elderly age in 3 study groups which are control group, zinc group and zinc + lysine group can be said same or no different from one way ANOVA test (p < 0.375). Previously, age characteristics were tested with Kolmogorov-Smirnov test and showed normal distribution and homogeneity of variance which showed significant with Levene test. The average age of respondents was 72.00 ± 9.15 years (group zinc + lysine), 75.38 ± 7.03 years (zinc group) and 77.00 ± 4.31 years (control group) with a range between 62 to 90 years old.

Twenty-two of twenty-four (22/24) or 91.7% of elderly respondents suffering from hypertension, which includes hypertensive patients with hypertension alone or accompanied by other degenerative diseases, such as diabetes mellitus, hypercholesterolemia, glaucoma, coronary heart disease, stroke, gastritis, osteoporosis and osteoarthritis.

Serum zinc level

The average of pre-test serum zinc levels was 88.64 ± 12.03 mg/dL for the control group, 88.34 ± 11.49 mg/dL for the zinc group and 87.84 ± 8.40 mg/dL for zinc + lysine group. There is no difference of pre-test serum zinc levels in all three groups ($p < 0.989$) (Table 1). Result of post-test serum zinc levels relatively higher than pre-test serum zinc levels. The average of post-test serum zinc levels was 86.92 ± 17.07 mg/dL for the control group, 93.32 ± 17.77 mg/dL for the zinc group and 102.98 ± 17.40 mg/dL for zinc + lysine groups. There is also no difference of post-test serum zinc levels in all groups ($p < 0.203$) (Table 1). Previously, from Kolmogorov-Smirnov test was found that data distribution on pre-test and post-test of three groups was normal, data variance is homogeneous.

Compared with the results of pre-test, there is an increase of post-test serum zinc levels in zinc group (4.98 µg/dL) and zinc + lysine groups (15.15 µg/dL) after supplementation but in the control group, serum zinc levels is decrease (-1.71 µg/dL). Increased of serum zinc levels which significant after supplementation only occurs in zinc + lysine groups ($p < 0.012$) (Table 1). There is no significant differences in changes of serum zinc levels (pre-test post-test) among three groups ($p < 0.273$) (Table 1).

There were no significant differences serum albumin levels in all three groups between before (pre-test) and after supplementation (post-test). Lysine syrup supplementation of 500 mg per day for 2 months in group zinc + lysine did not increase serum albumin levels. Examination results of post-test and pre-test albumin serum level indicates normal albumin levels with average range between 4.03 ± 0.32 g/dL to 4.20 ± 0.26 g/dL.

CD4 count

The control group had the highest CD4 count pre-test (878.38 ± 331.59 cells/ml) and CD4 count pre-test of zinc group were 713.63 ± 188.64 cells/ml. CD4 count pre-test of zinc + lysine group is the lowest with 545.13 ± 186.27 cells/ml. After comparison, there were no significant differences CD4 counts pre-test in three groups ($p < 0.042$) (Table 2). With the Post Hoc test, it is known that there is significant difference between control group with zinc + lysine groups.

After supplementation, the highest average of CD4 count is in zinc group (681.00 ± 170.93 cells/ml), followed by control group (658.63 ± 327.47 cells/ml). The lowest CD4 count in post-test was in zinc + lysine group with 549.50 ± 176.25 cells/ml (Table 2).

When compared with the results of pre-test, there is a decrease of CD4 counts in the control group (-219.75 cells/ml) and zinc group (-32.63 cells/ml). There was a slight increase in the zinc + lysine group (4.38 cells/ml), but the increase was not significant ($p < 0.784$) (Table 2). Because there is a difference CD4 counts pre-test among the three groups, the results of CD4 count in post-test cannot be compared, which can be compared is a change or difference of pre-test and post-test results. With Post Hoc test, there are significant differences in CD4 count changes (pre-test and post-test) between control group and zinc group and between control group with zinc + lysine group with one- way ANOVA test ($p < 0.000$) (Table 2). There is no significant difference in CD4 count change between zinc group and zinc + lysine group.

Group	Pre-test	Post-test	p-value (pre-test and post-test)	Change (pre-test- post-test)
Control	88.64 ± 12.03	86.92 ± 17.07	0.786	-1.71 ± 17.17
Zinc	88.34 ± 11.49	93.32 ± 17.77	0.625	4.98 ± 27.58
Zinc + Lysine	87.84 ± 8.40	102.98 ± 17.40	0.012	15.15 ± 12.78
p-value (between groups)	0.989	0.203		0.273

Table 1: Differences of pre-test and post-test serum zinc levels (µg/dl) in elderly respondents at "Surya" nursing house. 2013.

Group	Pre-test	Post-test	p-value (pre-test and post-test)	Change (pre-test- post-test)
Control	878.38^{b} ± 331.59	658.63 ± 327.47	0.001	-219.75^{a} ± 110.31
Zinc	713.63^{ab} ± 188.64	681.00 ± 170.93	0.445	-32.63^{b} ± 113.95
Zinc + Lysine	545.13^{a} ± 186.27	549.50 ± 176.25	0.784	4.38^{b} ± 43.42
p-value (between groups)	0.042			0.000

Table 2: Differences of CD4 count (cells/ml) before and after supplementation in elderly respondents at respondents at "Surya" nursing house. 2013.

Frequency of Infections in 2 Months	Control Group		Zinc Group		Zinc + Lysine Group	
	n	%	n	%	n	%
No one	3	37.5	7	87.5	6	75.0
1X	3	37.5	1	12.5	2	25.0
2X	2	25.0	0	0.0	0	0.0
Total	8	100.0	8	100.0	8	100.0
p-value				0.065		

Table 3: Frequency distribution of infections per person during supplementation in elderly respondents at "Surya" nursing house 2013.

Infection during supplementation

The highest number of elderly respondents who were exposed with infection is in control group with 5 people (62.5%), followed by zinc + lysine group with 2 people (25%) and the smallest is zinc group, only 1 (12.5%). After tested with Kruskal Wallis test, it turns out that the difference was not significant ($p < 0.065$) (Table 3).

The highest total incidence of infection during 2 months supplementation is in control group with 7 pain incidences because there are 2 people who each experienced 2 times infections and 3 people who each experienced 1 infection in 2 months. Next up is zinc + lysine group with 2 pain incidences and zinc group with 1 pain incidence in 2 months. From total 10 infection cases that affects elderly respondents for 2 months supplementation with zinc + lysine or zinc alone or placebo, type of infection disease are 5 cases of influenza, followed by 4 cases of diarrhea and 1 case of pharyngitis (Table 4).

Types of Infections Diseases	Control Group		Zinc Group		Zinc + Lysine Group	
	n	%	n	%	n	%
Diarrhea	4	42.9	0	0.0	0	0.0
Influensa	0	0.0	1	100.0	1	50.0
Pharyngitis	3	57.1	0	0.0	1	50.0
Total	7	100.0	1	100.0	2	100.0

Table 4: Types of infectious diseases that affects elderly during supplementation in elderly respondents at "Surya" nursing house 2013.

Discussion

Serum zinc level

Serum zinc levels of elderly respondents during pre-test are below normal (88.27 ± 10.29 μg/dL), accordance with the results of other studies in healthy elderly group in Italy which is about 88.5 ± 5.8 μg/dL, lower than serum zinc levels in young adults group (20-30 years) (120.0 ± 6.5 μg/dL). Pre-test serum zinc levels (before supplementation) in our study is lower than similar studies in Detroit, the United States where it was found that zinc serum levels in healthy elderly was 94.3 ± 11.4 μg/dL. Approximately, 35% of healthy elderly respondents in that study suffering from zinc deficiency by serum zinc levels less than 90 μg/dL [10]. Whereas in our study, the criteria of zinc deficiency is serum zinc levels were less than 70 μg/dL [18]. Elderly respondents in our study who experienced marginal zinc deficiency (zinc serum levels were less than 90 μg/dL) are 15 people (62.5%) that are greater than the results of research in Detroit, USA [10]. That differences could be due to the average age of the elderly respondents in our study were 10 years older. Zinc levels tend to decrease with increasing age [9]. It can also be caused by different diets.

After supplementation, there are increased serum zinc levels in zinc group and zinc + lysine groups but found that statistically, significant improvement only in zinc + lysine groups (15.15 ± 12.78 μg/dL) ($p < 0.012$). There are increased of serum zinc levels in zinc group too after supplementation, but not statistically significant. In the control group serum zinc levels is decrease.

In research in Detroit, USA, after supplementation of zinc tablets 45 mg per day (zinc gluconate) for 1 year in 49 healthy elderly, there is an increase of serum zinc levels (11.1 ± 7.23 μg/dL) ($p < 0, 0002$) but serum zinc levels in control group is decreased [10]. Other research in New Jersey, United States, using 103 healthy elderly people who were divided into 3 groups, the control group, the group which given 15 mg zinc tablets (zinc acetate) per day and the group which given 100 mg tablet zinc (zinc acetate) per day, everything is given for 3 months. Significant increase of serum zinc levels only in the group which receiving zinc tablets 100 mg per day (γ.7 ± 1.5 μmol/L) ($p < 0.05$). While the increase of serum zinc levels in the group which receiving 15 mg zinc tablets per day is only about 0.3 ± 0.8 μmol/L and insignificant [22]. In similar studies in Indonesia, after supplementation of zinc tablets 15 mg per day for 28 days in 30 healthy elderly women, there is an increase of serum zinc levels in β7 people (90%) with average 4.54 ± γ.84 μmol/L ($p < 0.001$) [23]. Have not found the results of studies using zinc and lysine supplementation simultaneously. In elderly, the amount of zinc that is absorbed through the small intestine decreases, which is about 17%, compared with young adults that their zinc absorption reaches 31% [24]. By giving lysine, zinc absorption in the elderly expected to increase. In experiments on mice, lysine can increase zinc absorption in the small intestine by forming a bond (ligand) with lysine, resulting in increased serum zinc levels [17].

CD4 count

After zinc and lysine supplementation increase CD4 counts found in zinc + lysine group (4.38 ± 43.42 cells/ml), but this increase was insignificant. Results of this study similar to other results of similar studies in Indonesia, which showed 36% of healthy elderly respondents has increased CD4 count (26.83 ± 137.46 cells/ml) ($p < 0.29$) after supplementation of zinc tablets 15 mg per day for 28 days [23].

There is Italian study that used 58 elderly people over 65 years in nursing home who were given supplements of zinc tablets 25 mg per day (zinc sulfate) for 3 months. The results showed significant increase of CD4 count ($p < 0.016$) and cytotoxic T-cells ($p < 0.005$), compared with control group and group that received vitamin A supplementation [25].

Results of research about lysine fortification on wheat flour in 40 families (240 people) in Pakistan showed the group that received lysine-fortified wheat flour has increased CD4 count about 2.4% to 3.7% and that increment is significant ($p < 0.05$) with estimated consumption of lysine about 2 to 2.5 grams per day. The control group experienced a decline in CD4 counts [13]. There is a similar study in China on 88 families who were given wheat flour that fortified with 3 grams of lysine per kilogram of flour for 3 months. At the end of study showed that there is an increase of CD3, C3, IgG, IgA and Ig M significantly in lysine group compared with the control group. CD4 count was not checked [14].

The increase of CD4 counts in zinc + lysine group may be related to the effect of lysine on the increase of zinc absorption because apparently the highest increase serum zinc levels is in zinc + lysine group as well. While the decline of CD4 counts in zinc and control group, as well as an increase of CD4 count which not significant in zinc + lysine group after supplementation may be due to the density of elderly respondents activity at research time that could reduce respondents rest time. In addition, inflammation factors in respondents because osteoarthritis and gastritis (7 people or approximately 29.2% of respondents) could be one of the factors that cause decrease of CD4 count. Factors that could affect CD4 cells levels except zinc and albumin level are method of examination, nutritional status, malnutrition, other micronutrient deficiencies such as vitamin A, vitamin B6, vitamin C, folic acid, vitamin E and selenium, race, gender, height of residence, physical activity, socio-economic status, circadian rhythms, some diseases especially inflammatory and infections and the use of certain drugs, especially immunosuppressive [3,26].

Majority respondents and infections were in control group. Group with the smallest respondents and infection is zinc group. The high of total infection in control group could be associated with large decrease of CD4 count in control group (-219.75 ± 110.31 cells/ml) and CD4 count post-test (658.63 ± 327.47 cells/ml). While in zinc + lysine group CD4 count was increased (4.38 ± 43.42 cells/ml), but the lowest post-test CD4 count is 549.50 ± 176.25 cells/ml. While the zinc group with the smallest total infections experienced a slight decline of CD4 count (-32 ± 17.71 cells/ml), but CD4 count post-test in zinc group is the highest (681.00 ± 170.93 cells/ml). It is said that ideal CD4 count for elderly to stay healthy is about 700 cells/ml [19].

Infections

Zinc supplementation can reduce the frequency of occurrence and duration of respiratory infections and diarrhea. These results are consistent with research in Detroit, USA, which total of infection that affects the control group (25 persons) for 1 year were 35 cases with average 1.4 ± 0.95 incidents per year and total of infection that affects the zinc group (24 persons) were 7 cases for 1 year with average 0.29 ± 0.46 incidents per year. Zinc supplementation dose that given is 45 mg per day for 1 year and the results are statistically significant ($p < 0.001$) [10].

Giving zinc tablets in elderly respondents on healing period at a hospital in Italy also showed a decrease in the frequency of infectious disease recurrence. After giving zinc tablets 12 mg per day for a

month, an increase of CD4 counts in elderly during the healing of chronic obstructive bronchitis disease happened (462 ± 48 cells/ml to 690 ± 41 cells/ml) ($p < 0.01$), so that frequency of recurrence or relapse also decreased. Two factors that support the occurrence of infections in elderly is zinc deficiency and low CD4 counts. The infection itself also causes decrease of zinc levels and CD4 counts [19].

In research about lysine supplementation in Ghana with 271 respondents (adults and children) showed decrease incidence and duration of illness (diarrhea and bronchopneumonia) in group which receiving lysine supplementation. Lysine dose given is 1 gram per day for 3 months [12]. Previous studies in Pakistan showed that there is no difference frequency of infection among group which given lysine with control group [13].

Type of infectious diseases that suffered by respondents during period of supplementation are influenza (5 cases), diarrhea (4 cases) and pharyngitis (1 case). Type of this infectious disease is rather similar with other studies in a nursing home in Belgium. Infections that affect many elderly in nursing home are respiratory infections including bronchopneumonia, gastroenteritis (diarrhea), urinary tract infections and skin infections [27].

Conclusion

Zinc and lysine supplementation may increase serum zinc levels significantly and increase CD4 counts, though not significant. The way it works through effect of increased zinc levels with the help of lysine to thymulin activation to improve the process of differentiation and maturation of T lymphocytes cells, including CD4. The increase of CD4 count could decrease incidence of infection, although it was not statistically significant. In general, zinc and lysine supplementation is better than zinc supplementation alone.

To know more detailed relationship between zinc, lysine and CD4, further research is needed with larger sample size and added a group that only received lysine supplements alone to determine the effect of basis single lysine for CD4 count. If possible, do an examined of thymulin activity factor as a between variable that connecting zinc levels with CD4 counts.

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