Effect of the purified filtrate of *Bacillus subtilis var. natto* culture on the lectin-like oxidized low-density lipoprotein receptor index

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Abstract

The lectin-like oxidized low-density lipoprotein receptor index (LOX index) is a biomarker for monitoring the progression of arteriosclerosis, and is calculated by multiplying the lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) ligands containing apolipoprotein B (LAB) and soluble LOX-1 (sLOX-1). We carried out a pilot study to evaluate the effect of the purified filtrate of *Bacillus subtilis* var. *natto culture* (NKCP), which is a functional food derived from "natto" (a traditional Japanese foodstuff of fermented soybeans), on the LOX index of patients with a high value. The LOX index was lower in more than half of the patients, although the results did not clearly show that NKCP lowered the LOX index. However, the lowering effect of NKCP on the LOX index was mainly due to the reduced sLOX-1. Therefore, NKCP may have anti-inflammatory effects.

Introduction

According to a survey by the Ministry of Health, Labor and Welfare of Japan, the number of patients suffering from cardiac disease was 1.73 million, and those from cerebrovascular diseases was 11.1 million in 2017. Both diseases are strongly associated with thrombus formation and blood flow insufficiency caused by arteriosclerosis. Arteriosclerosis progresses slowly and is rarely accompanied by subjective symptoms. Therefore, many patients are unaware of problems affecting their vascular system until the onset of serious diseases such as acute myocardial infarction, myocardial infarction, and cerebral infarction.

Arteriosclerosis is an inflammatory disease that results from the progression of vascular endothelial insufficiency. When LOX-1 ligands containing apolipoprotein B (LAB) are produced by low-density lipoprotein (LDL) oxidization due to various factors, they bind to lectin-like oxidized LDL receptors (lectin-like oxidized LDL receptor-1, LOX-1) and cause a variety of reactions. LOX-1 is partly cleaved on the cell membrane and released into the blood in the form of soluble LOX-1 (soluble LOX-1, sLOX-1). The LOX index, which is calculated by multiplying LAB and sLOX-1, is used as a biomarker for monitoring the progression of atherosclerosis. Epidemiological studies that tracked about 2,500 patients over 11 years, reported a three-fold higher cerebral infarction incidence rate, and twice as high myocardial infarction incidence rate was recorded in the high LOX index (7160 or above) group than in

the low LOX index (less than 1068) group.

NKCP, a functional food derived from *Bacillus subtilis* var *natto*, has been shown to inhibit thrombus formation and promote thrombolysis in animal experiments[2]. In addition, a human double-blind test demonstrated that this substance lowered blood pressure[3], improved stiff neck and lower back pain[4], alleviated muscle stiffness, and enhanced skin temperature[5].

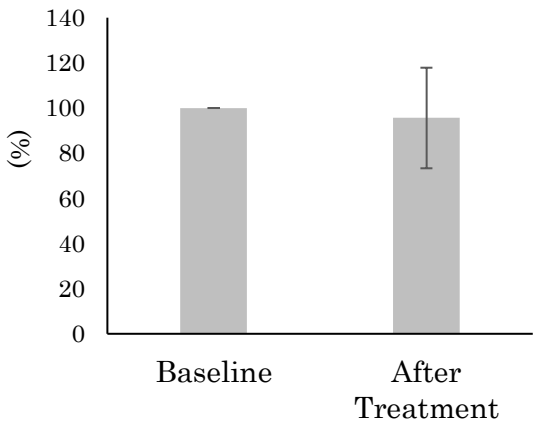
This pilot study was carried out to evaluate the effect of NKCP on the LOX index of elderly patients with higher values.

Methods

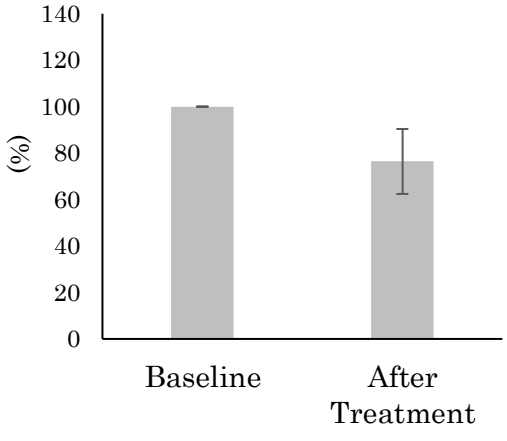
The study was designed as an open study on 8 patients (5 males, 3 females; aged 49 to 74 years old, with an average age of 62.3) with higher values of LOX index (7160 and above). They were administered 2 to 8 tablets of NKCP per day, and their LOX index values were measured again 2–5 months later. The baseline value was set at 100, and the rate of change was evaluated using a t-test. The patients were given sufficient information about the trial in advance and a written informed consent was obtained. During the trial period, the patients continued taking medicines, and there were no change in their daily activities, which may affect the results of the trial, such as exercise.

Results

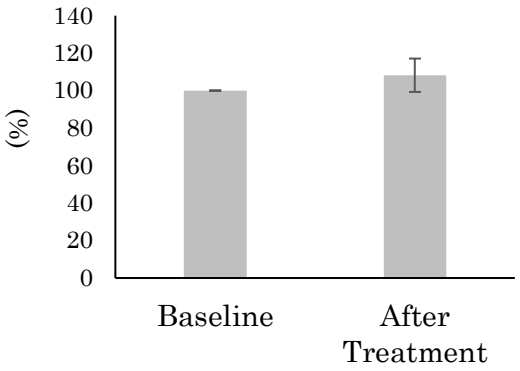
Before NKCP administration, the average LOX index was 28966.1, with the lowest at 7416, and the highest at 73593. The values varied greatly among the individual patients. Therefore, we focused on the rate of change among these values, before and after NKCP administration. The LOX index, sLOX-1, and LAB before administration were set to 100. The rates of change are shown in Fig.1, panels A to C. Although no statistical significance was observed, NKCP administration resulted in a slight decrease in LOX index, reduction in sLOX-1, and slight increase in LAB.



A. The rate of change in LOX index of 8 patients
Vertical bar = SE。 $p = 0.873$



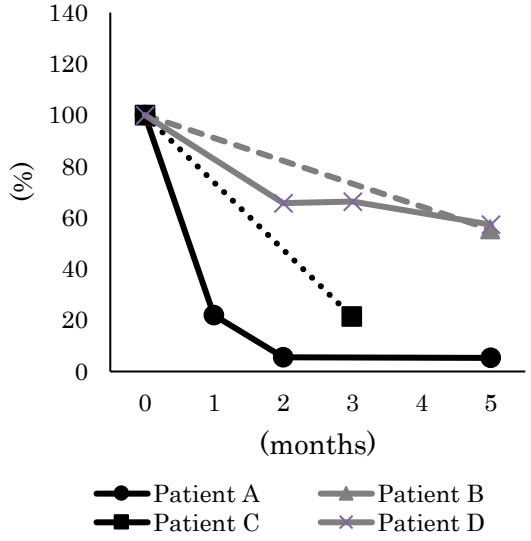
B. The rate of chang in sLOX-1of 8 patients
Vertical bar = SE, $p = 0.201$



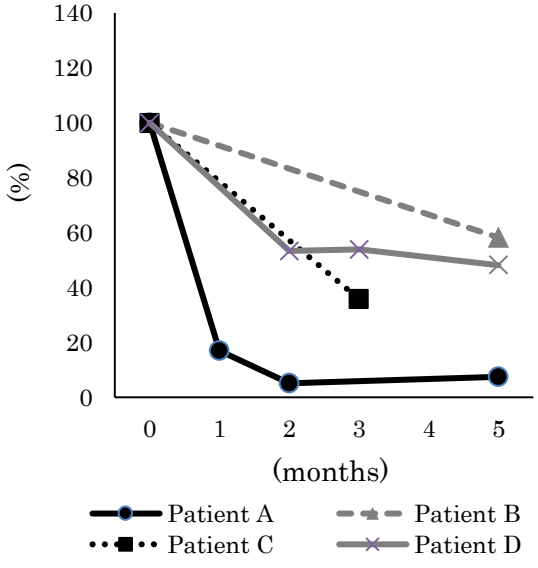
C. The rate of change in the LAB of 8 patients
Vertical bar = SE, $p = 0.467$

Fig. 1 The rate of change in LOX index, sLOX-1, LAB of 8 patients

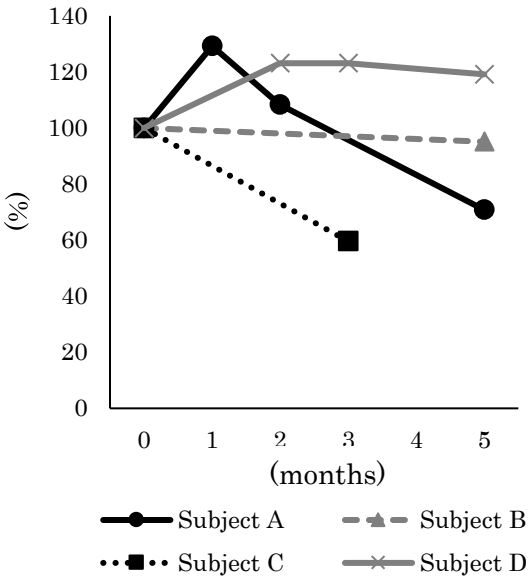
Of the eight patients, the LOX index decreased after NKCP administration in 4, half of all patients (2 males, 2 females; aged 50 to 74 years, with an average age of 64.8 years) (Fig. 2-A). When we focused on the data of four patients whose LOX index decreased after NKCP administration, 3 out of the 4 patients could be categorized as being out of the high value group, since their values reduced below 7160. Patient A was administered 4 tablets per day for the first month, and his LOX index greatly decreased. His dosage was reduced to 2 tablets per day after the second month, which was continued for five months. This value further declined in the second month and was subsequently maintained. Patient B was administered NKCP at 2 tablets per day, while patients C and D were administered 4 tablets per day. The LOX index was calculated by multiplying sLOX-1 with LAB. The decreased LOX index in the four patients may be greatly associated with lowered sLOX-1 (Figure 2-A to C). Although this study's results are limited due to its small sample size, it is an important finding that NKCP eminently deceased the LOX index.



A. The rate of change in LOX index of 4 patients



B. The rate of change rates of LOX-1 in 4 patients



C. The rate of change in LAB of 4 patients

Patient A : 70 years old, male, NKCP intake at 4 tablets/day from baseline through 1 months, and 2 tablets/day from 2 through 5 months.

Patient B : 74 years old, male, NKCP intake at 2 tablets/day from baseline through 5 months.

Patient C : 50 years old, female, NKCP intake at 4 tablets/day from baseline through 3 months.

Patient D : 65 years old, female, NKCP intake at 4 tablets/day from baseline through 5 months

Fig. 2 Change rates of LOX index, sLOX-1, and LAB in 4 four patients

Discussion

We could not clearly demonstrate that NKCP decreased the LOX index in this study. The effect of NKCP on the LOX index of each patient varied; however, the LOX index decreased in four patients, and half of the patients had a high LOX index (7160 and above). In addition, the index was reduced to below 7160 in three patients.

The LOX-1 ligand containing apoB (LAB) is involved in the progression of atherosclerosis. In addition to oxidized LDL, LAB is known as an index for alondialdehyde, acetylation, carbamination, saccharification, and negative charge electrification. LAB may also increase due to smoking, diabetes, chronic kidney disease, and cerebral infarction. Although LOX-1 is less expressed in healthy individuals, it significantly increases in patients with hypertension, dyslipidemia, and diabetes, which leads to arteriosclerosis. sLOX-1 is produced when LOX-1 present in vascular endothelial cells is isolated by protease and released into the blood. sLOX-1 is also increased by stimulation with LAB and cytokines. LAB is decreased by antioxidants, while sLOX-1 is reduced due to its anti-inflammatory effect[6]. As shown in the figures, the effect of NKCP on the lower LOX index is mainly attributed to reduced sLOX (Figure 2-A–C). This suggests that NKCP may possess anti-inflammatory effects. This study is interesting because NKCP has not been shown to exert anti-inflammatory effects in previous studies, although NKCP has been reported to inhibit thrombus formation, improve stiff neck and lower back pain, improve limb coldness and headache, and lower blood pressure.

Conclusions

This study did not clearly demonstrate that NKCP decreased the LOX index. However, the

effect of NKCP on the LOX index varied among patients, and a decreased index was observed in half of all patients. The effect of NKCP on the lower LOX index is mainly attributed to reduced sLOX-1, suggesting an anti-inflammatory effect of NKCP. We suggest that people with a higher LOX index should take 2 to 4 tablets of NKCP per day for the first month, and measure their LOX index. They should continue NKCP intake even when a decreased index is observed.

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