Journal of Hemorheology Research 5 (1), 2002

Improvement of Blood Fluidity using NKCP, a Dried Culture Filtrate of Bacillus Subtilis

Tetsuya Hayashi (Daiwa Pharm), Chie Takahashi (Microchannel Array Technology Team), and Yuji Kikuchi (Microchannel Array Techology Team). -

ABSTRACT

NKCP, a nattokinase containing food product without the distinctive flavor of natto, was prepared from the culture filtrate of Bacillus subtilis. To confirm the effect of this food extract on blood fluidity, NKCP was given orally to 13 healthy volunteers for 1 week. Fibrinolytic activity was determined by euglobulin lysis time, and this study showed that those subjects given 250-500mg/day of NKCP had increased fibrinolytic activity. One healthy volunteer repeatedly took 1g of NKCP after each meal and the whole blood passage time, determined using MC-FAN, was markedly reduced after 7 days of ingestion.

INTRODUCTION

In Japan, the number of patients with thrombosis, such as ischemic heart diseases and cerebral infarction, has significantly increased, resulting in increasing focus being given to prevention. Natto is a traditional Japanese food that has well been known to have beneficial effects on health. Sumi et al. discovered serine protease with high fibrin degrading activity in natto, which he named nattokinase(1), and confirmed that oral administration of nattokinase gradually increases fibrinolytic activity(2). Since the discovery of nattokinase, natto has been regarded as a functional food that can help protect against thrombosis. However, many people do not like natto because of its distinctive flavor and texture, so in order to make the benefits of natto more widely available, a food-based powder called NKCP was developed, and its ability to optimize blood fluidity upon oral ingestion was confirmed.

METHOD

Preparation of NKCP

Bacillus subtilis was cultured in a liquid medium containing soybean protein as the main component. Low-molecular components were then removed from the culture filtrate by ultrafiltration, and the filtrate was freeze-dried. The dried culture was powdered, mixed with a filler – dextrin – and then regulated for consistent nattokinase activity. The resulting powder was labeled NKCP.

Confirmation of Increased Fibrinolytic Activity

Subjects were 13 healthy volunteers aged 20-60 in the company. Gelatin hard capsules were filled with NKCP and then coated to make them acid resistant. The subjects stopped eating natto from 7 days before the start of the study and also underwent blood sampling just before the first NKCP ingestion. Once started on NKCP, their daily dose was 500 mg/day (8 subjects) and 250 mg/day (5 subjects), with the NKCP being taken once a day after supper. Blood was sampled at 9:00 a.m. on Days 4 and 7 after the start of NKCP ingestion, and the euglobulin lysis time was measured.

Confirmation of Blood Fluidity Improving Effect

The subject was 1 healthy male staff member in the company (39 years old). Gelatin hard capsules were filled with NKCP and then coated to make them acid resistant. The subject underwent whole blood passage time measurement every several days from 1 month before the start of NKCP ingestion. The dose was 3 g/day and 1 g was taken after each meal. The whole blood passage time was measured at about 10:00 a.m. on Days 5, 7, 12, and 14 after the start of ingestion, using a blood fluidity analyzer MC-FAN, according to Kikuchi et al.'s method (3).

RESULTS

Regarding the effect of NKCP ingestion on fibrinolytic activity, the euglobulin lysis time reduced in 7 out of 8 of the subjects on Day 4 of ingestion and in all subjects on Day 7 in the 500 mg/day group. The average difference in euglobulin lysis time between before and after ingestion was 1.1 hours on Day 4 and 1.6 hours on Day 7, as shown in Fig. 1. A similar reduction was observed in 3 of 5 subjects in the 250 mg/day group.

Regarding the effect on blood fluidity, the whole blood passage time markedly reduced on Day 7 of NKCP ingestion, as shown in Fig. 2. The mean passage time was 54.8 seconds for 1 month before the start of ingestion and 46.7 seconds between Days 7 and 14 of ingestion. The mean passage time reduced by about 8 seconds.

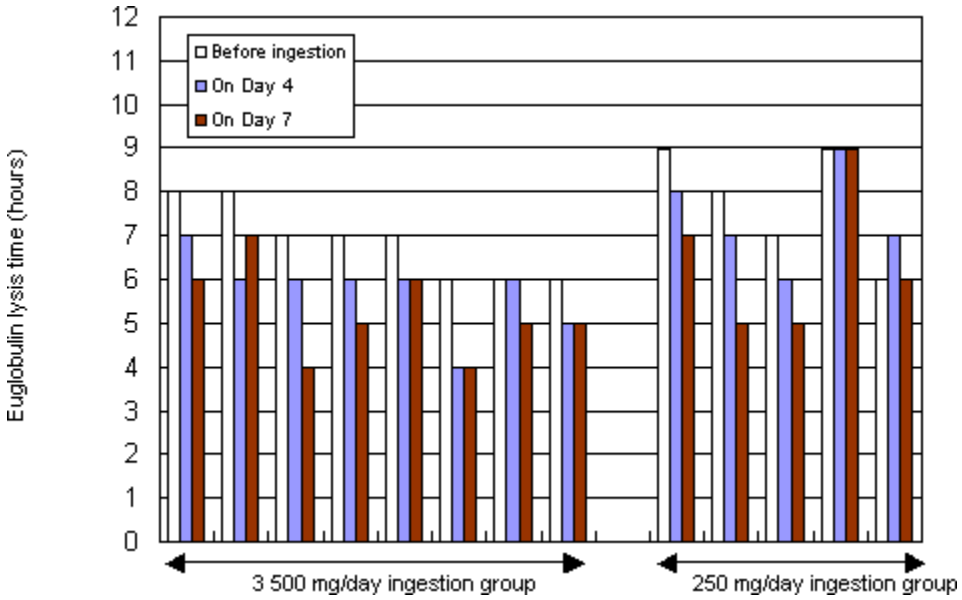

Figure 1: Changes in euglobulin lysis time in subjects given NKCP orally

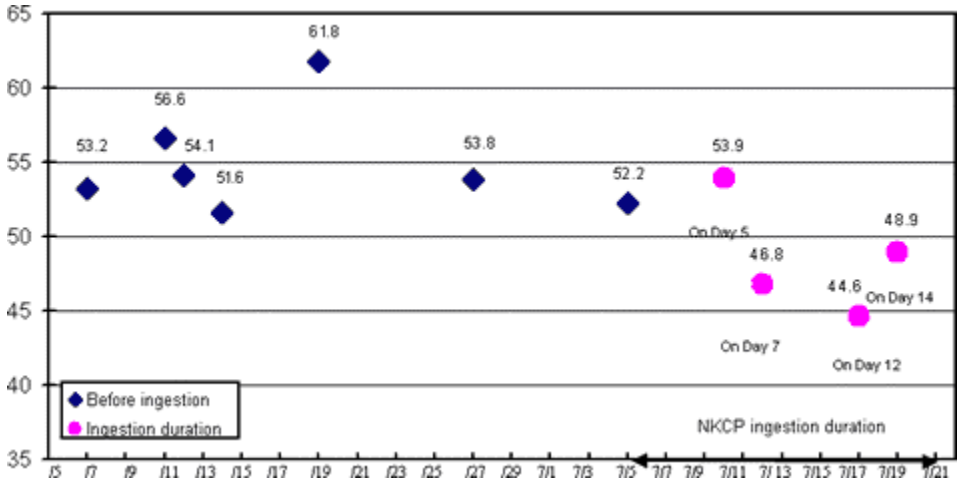

Figure 2: Changes in whole blood passage time in subjects given NKCP orally

DISCUSSION

NKCP is produced by removing low molecular components from the culture filtrate of Bacillus subtilis and drying and powdering the resulting culture filtrate. It contains soybean protein, its degradation products, and high amounts of nattokinase, but without the distinctive flavor of natto. Favorable results were obtained in mutagenicity, acute toxicity, subacute toxicity, antigenicity, and excessive ingestion studies, showing that NKCP is a promising functional food for the prevention and improvement of thrombosis. In the present study, oral ingestion of NKCP at 500 mg/day or 250 mg/day clearly reduced the euglobulin lysis time, confirming that it was able to increase fibrinolytic activity. Also, measurement using a blood fluidity analyzer (MC-FAN) showed that oral ingestion of NKCP reduced the whole blood passage time, suggesting that NKCP inhibits platelet aggregation and thus reduces the probability of thrombus formation.

REFERENCE

Sangenchaya 1-16-19, Setagaya-ku, Tokyo 154-0024

Kan-nondai 2-1-12, Tsukuba-shi, Ibaraki 305-8642

Yuji Kikuchi, Chieko Takahashi, and Atsuko Isono: Determination Using MC-FAN and Comparison between Men and Women of Whole Blood Passage Time, Journal of Hemorheology Research 2, 25-28 (1999)