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Research Article

Nattokinase Positively Impacts Cardiovascular Health through Inhibiting Platelet Aggregation and Positively Effecting Blood Coagulation

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Abstract

Cardiovascular disease is among the leading causes of death worldwide. Many conditions such as myocardial infarctions and strokes are caused by the blockage of blood flow which can be due to an imbalance in clotting homeostatic mechanisms. Interventions to prevent and address the imbalances while minimizing side effects are desirable. One such option is nattokinase, a phytochemical derived from soybeans. It addresses clotting via platelet aggregation and fibrinolytic actions without apparent negative side effects. *In vivo* and *vitro* studies have found potent fibrinolytic activity of nattokinase. Rodent studies reported that it is four times more effective than plasmin in dissolving a thrombus. Human studies have reported that oral nattokinase supplementation lead to thrombolysis and increased anti-coagulation factors. In addition, supplementation of nattokinase significantly decreased levels of fibrinogen, factor VII and factor VIII in subjects with cardiovascular disease. Nattokinase also has been shown to ameliorate blood viscosity, which lowers risk for cardiovascular disease. It is the purpose of this paper to review the studies related to nattokinase supplementation for its impact on reducing platelet aggregation and enhancing fibrinolytic activity in maintaining homeostatic balance.

Keywords: *Natto; Nattokinase; Fibrinolytic; Blood Viscosity; Circulation; Thrombosis; Platelet Aggregation; Thrombus*

Abbreviations

FU; Fibrinolytic Units; CV: Cardiovascular; TPA: Tissue Plasminogen Activator; kDa; Kilodalton

Introduction

Cardiovascular disease is among the major causes of death worldwide. Conditions such as acute myocardial infarction, ischemic heart disease and stroke are often caused by the blockage of blood flow. The potential blockage can be caused my many factors. Regardless, removal or prevention of the clot is important for the prevention and management of such thrombolytic conditions. This is often done via pharmacological or surgical interventions, though many do not address an already formed thrombus [1]. This approach to management is logical when one considers that normal functioning of the circulatory system is achieved and maintained via a delicate hemostatic balance between fibrin formation and degradation. This homeostatic balance allows for clotting when needed such that platelet aggregation will occur upon injury to reduce blood flow, coagulation will occur through a series of reactions to prevent "bleeding out" and finally the clot will be removed by fibrinolysis when the healing has occurred to restore healthy blood circulation. The balance can be restored if the clot is removed by plasmin, often without incident. However, when there is an accumulation of fibrin that exceeds the ability of plasmin to restore the balance a thrombus is formed that can block blood flow and thus oxygen supply causing tissue damage, ultimately leading

to thrombolytic diseases such as acute myocardial infarction and stroke [2]. Therefore, an intervention that can both prevent formation of a clot by targeting platelet aggregation and could dissolve an existing thrombus by enhancing fibrinolytic activity, but without the side effects often associated with pharmacological interventions, such as bleeding, would be advantageous.

Fermented soybean, also known as natto contains natural phytochemicals that have biological activity in animals and humans. The most prevalent phytochemical is nattokinase. Nattokinase itself is a serine-proteinase comprised of 275 amino acids and has a molecular weight of 27.7 kDa [3]. Nattokinase has been reported to have strong fibrinolytic activity [4]. Nattokinase is absorbed along the intestinal tract with high bioavailability and is considered pH and temperature stable.

In vitro studies have found potent fibrinolytic activity of nattokinase [3,5,6]. Rodent studies have found a four-factor effect of nattokinase as compared to plasmin for dissolving a thrombus (blood clot) [4,6]. An *in vivo* study reported positive thrombolytic effects of nattokinase in a carrageenan induced rat model of thrombosis. A follow-up human study conducted in healthy Japanese subjects found that oral nattokinase supplementation potentiated thrombolysis and anti-coagulation factors [7]. Further, a more robust human study that included healthy subjects, individuals with cardiovascular disease risk factors and those on dialysis, found oral nattokinase supplementation significantly decreased levels of fibrinogen, factor VII and factor VIII, indicating a potential utility for cardiovascular disease [8].

Purpose of the Study

The purpose of this paper is to review the *in vitro, in vivo* and applied studies with nattokinase with focus on the physiologic and biologic impacts over time in healthy and non-healthy populations.

Materials and Methods

A literature search using the terms nattokinase, oral nattokinase, and nattokinase supplementation was conducted in PubMed and Google Scholar (September 3, 2019, completed October 1, 2019). In addition, data from Japan Bio Science Laboratory (JBSL) was also reviewed. JBSL has supplied nattokinase (as NSK-SD®) for clinical characteristic and outcome studies.

Results and Discussion

In Vitro

The effects of nattokinase on *in vitro* platelet aggregation as compared to aspirin were assessed to determine the mechanism of action. Platelet aggregation was measured by an aggregometer using the turbidimetric method. Four hundred (400) ug/ml nattokinase or 200 ug/ml aspirin were utilized for the impact comparisons because it is a standard recommended therapy for those with cardiovascular disease. Nattokinase was found to significantly inhibit platelet aggregation induced by collagen (a standard *in vitro* method) by ~40%. During the collagen-induced platelet aggregation, thromboxin B_2 (TXB_2) formation was inhibited by nattokinase in a dose dependent manner (20% to 37.5% inhibition at 10, 100 and 1000 ug/ml). The response to high dose nattokinase (1000 ug.ml) induced an effect similar to that of aspirin (35.8%). The data indicated that nattokinase can inhibit blood clots triggered by thrombin. In addition, it was noted that thromboxin A_2 (TXA_2) adhesion to platelets was also inhibited. Collectively, these results infer that the effects of nattokinase are similar to that of aspirin. The researchers concluded that due to these effects, nattokinase can have a positive effect on blood flow [9].

Nattokinase acts as a direct anti-fibrinogen agent and does not appear to destroy the fibrinogen compound. In an *in vitro* study, NSK-SD was evaluated for its coagulation profile. The dose of nattokinase and NSK-SD examined was 4000 fibrinolytic units (4000 FU), which was later diluted to have working concentrations of 0, 1, 2, 4, 8, 16, 100 and 200 FU/ml. The tested plasma utilized a range of nattokinase/NSK-SD, from 0, 0.1, 0.2, 0.4, 0.8, 1.6, 10 and 25 FU/ml. The effects of the nattokinase/NSK-SD on the coagulation profile comprised of fibrinogen activity, fibrinogen antigen, thrombin clotting time, reptilase time, tissue plasminogen activator antigen (tPA), plasminogen activator inhibitor-1 (PAI-1) activity, alpha-2antiplasmin, D-dimer, fibrinogen degradation products, euglobulin clot lysis time and amidolytic

activity was evaluated. In summary, nattokinase/NSK-SD elicited the strongest fibrinolytic effects on fibrinogen and to a lesser degree, on fibrin too. Clotting ability was not impacted. Tissue plasminogen activator was not influenced, plasminogen activator inhibitor-1 (PAI-1) was increased, and fibrin degradation products were detected [10].

Nattokinase was evaluated to determine how it influences fibrinolytic activity. In a dose dependent manner nattokinase enhanced tissue-type plasminogen activator-induced fibrin clot lysis. The enhancement seems to be due to direct fibrin dissolution by nattokinase. This mechanism appears intrinsic for potentiating fibrinolysis [3].

A preliminary *in vitro* experiment assessed the effects of nattokinase on red blood cell aggregation and whole blood viscosity profiles. Results indicated significant, dose-dependent decreases of erythrocyte aggregation with enzyme treatment: at the activities employed (i.e. 15.6, 31.3, 62.5 and 125 units/ml), mean decreases from control were 21.9%, 25.9%, 49.7% and 62.0%, indicating that nattokinase diminished RBC aggregation in a dose dependent manner, similar to those reported in animal studies [11]. Reduced blood viscosity was also associated with low shear viscosity [12].

In vivo

In a rodent model, the thrombolytic effect of nattokinase was tested. More specifically, the impact of nattokinase was evaluated for its ability to reopen the common carotid artery after a chemically (acetic acid) induced injury. The resulting occlusive thrombosis was caused by platelet aggregation. Additionally, data revealed treatment with urokinase or tissue plasminogen activator (tPA) restored blood flow within 60 minutes. Following the proof that the model was effective for evaluating ingredients that may have a thrombolytic effect, researchers examined the effects of nattokinase as compared to plasmin and elastase. The results indicated that the agents enhanced arterial blood flow by 0.62 ± 5.3%, 15.8 ± 0.7% and 0% respectively. These results indicated that nattokinase has greater thrombolytic activity than plasmin and elastase. In fact, at the lowest dose of nattokinase tested (0.02 umol/kg), there was an effect on recovery of blood flow at the 60-minute post use time-period (17.7 ± 5.0 vs. 15.8 ± 4.5%), similar to plasmin but superior to elastase (0%). It appears that nattokinase is less sensitive to the cleavage of fibrinogen. The study found nattokinase to be a strong thrombolytic agent *in vivo* [11].

A human single dose study was conducted using 200 grams of natto in 12 healthy subjects. The study compared and examined the effects of nattokinase on the duration of euglobulin lysis time (ELT) and euglobulin fibrinolytic activity (EFA). The results demonstrated a shortening of the ELT and an enhancement of plasma fibrinolytic activity. The single dose of nattokinase enhanced the plasma fibrinolytic activity for up to 8 hours. Multiple days of dosing (1.5 gm, three times per day) for eight days resulted in a consistent effect on whole blood clot lysis time. This was accompanied by a gradual, but significant effect on EFA by the eighth day ($p < 0.02$). Tissue plasminogen activator (TPA) was significantly elevated from pre-dose levels by the fourth day, which remained significant as well on day eight ($p < 0.05$). TPA as a medicine has been developed and is used as a pharmaceutical intervention against potential thrombi or embolism because of its affinity to fibrin deposits. In this study, oral nattokinase had consistent effects across a few, but very important fibrinolytic enzymes, indicating its utility as a natural means to reduce risks of a thrombus [13].

An open-label clinical trial that included three groups of subjects (healthy, cardiovascular disease risk factors and those on dialysis) evaluated the impacts of 4000 FU nattokinase per day for two-months. Outcome objectives across all groups included measuring changes in fibrinogen, factor VII and factor VIII in adult humans over the two-month period. Each cohort included 15 subjects (45 total). A significant time effects was found, but not a difference between the groups (no group effect). Irrespective of the group, there was a significant impact on fibrinogen ($p = 0.03$), factor VII ($p < 0.01$) and on factor VIII (p, 0.001). The decrease in fibrinogen, factor VII and factor VIII was 9, 14 and 17 percent, respectively. These results indicated that oral nattokinase can positively impact cardiovascular disease biomarkers [8].

To assess the effects of nattokinase on platelet aggregation 10 healthy adult subjects were give 4000 FU of nattokinase, the equivalent of 2 packets of commercially available natto. Platelet aggregation was measured before and after 3, 6, 12, 20 hours after administration.

Plasma aggregation efficacy was measured by Platelet Aggregation Analyzer (PA 20), Platelet Aggregation Analyzer. Inhibitory effect was observed in all subjects [14]. In a similar study, nine subjects with spontaneous platelet aggregation caused by smoking or hyperlipidemia were given 4000 FU of nattokinase and a blood sample was taken six hours after administration and tested for platelet aggregation. There was a strong inhibitory effect on platelet aggregation and there was inhibition of spontaneous aggregation in most of the subjects. Additionally, the rate of small aggregates increased, whereas the medium and large aggregates decreased. The authors hypothesized that this was due to nattokinase suppressing the small aggregates from becoming larger thus inhibiting spontaneous aggregation which has been associated with smoking or diabetes [15]. Together, these open label studies suggest that nattokinase can decrease platelet aggregation with one dose in healthy subjects and in those who smoke or have hyperlipidemia.

This effect was further supported by a double-blinded, placebo-controlled, cross-over trial in 12 healthy subjects evaluated the effects of a single dose of 2000 FU nattokinase or placebo on coagulation and fibrinolysis parameters. After dosing, subjects had blood withdrawn over an eight-hour period and evaluated for change from pre-dose baseline for physiologic effect or impact. As a result, D-dimer concentrations at 6, and 8 hours, and blood fibrin/fibrinogen degradation products at 4 hours after NK administration elevated significantly ($p < 0.05$, respectively). Factor VIII activity declined at 4 and 6 hours ($p < 0.05$, respectively), blood antithrombin concentration was higher at 2 and 4 hours ($p < 0.05$, respectively), and the activated partial thromboplastin time prolonged significantly at 2 and 4 hours following nattokinase administration ($p < 0.05$ and $p < 0.01$, respectively). This study demonstrated that a single dose of nattokinase administration enhanced fibrinolysis and anti-coagulation via several different pathways simultaneously. Hence oral nattokinase demonstrated consistent positive effects for cardiovascular health [16].

Similarly, a randomized double blind, placebo-controlled trial in adults with hypercholesterolemia evaluated nattokinase 2,000 FU dose as compared to placebo for impacts over an eight-week period on hemostatic factors; collagen-epinephrine closure time (C-EPI CT) prothrombin time (PT) and activated partial thromboplastin time (aPTT). After 8 weeks of treatment, the nattokinase group exhibited significant increases in C-EPI CT, PT, and aPTT. The nattokinase group showed significantly greater increases in C-EPI CT ($p = 0.001$) and aPTT ($p = 0.016$) than the placebo group. Moreover, at eight weeks, the nattokinase group showed a significantly higher C-EPI CT than the placebo group ($P = 0.001$). Additionally, a significant correlation between PT and PTT was observed ($r = 0.491$, $P < 0.001$). In summary nattokinase supplementation was associated with prolonged C-EPI CT and aPTT in nondiabetic hypercholesterolemic subjects. Nattokinase at a dose of 2,000 FU improved blood flow.

Conclusion

In conclusion, there is a long history of oral ingestion of nattokinase via fermented soybean as natto. The safety profile of nattokinase has been established from historical use coupled with pre-clinical and human outcome studies. It has been reported that Nattokinase improved blood flow based on its fibrinolytic and antithrombotic effects. More specifically, it helps to address thrombus formation via its effects on platelet aggregation which occurs at the beginning of clot formation as well as by promoting fibrinolytic activity to restore healthy blood flow once a thrombus has formed. The data supports that nattokinase improves blood flow and blood viscosity while reducing cardiovascular disease risk factors in healthy subjects as well as those with hypercholesterolemia, diabetes and for those on dialysis. Eating natto or supplementing the diet with nattokinase (as NSK-SD®) may be considered as a lifestyle modification for promoting cardiovascular and overall health [17].

Conflict of Interest

The authors declare that they received an unrestricted writing grant from Japan Bio Science Laboratory (JBSL) for the writing of this manuscript.

Bibliography

1. Kumar SS SA. "Fibrinolytic Enzymes for Thrombolytic Therapy". In: N L, ed. Therapeutic Enzymes: Function and Clinical Implications. Advances in Experimental Medicine and Biology. Volume 1148. Singapore: Springer (2019).

2. Arnout J HM and Lijnen HR. "Haemostasis". In: Moncada S HA, ed. The vascular endothelium II. Handbook of experimental pharmacology. Volume 176/II. Berlin: Springer (2006): 1-41.

3. Urano T., *et al*. "The Profibrinolytic Enzyme Subtilisin NAT Purified fromBacillus subtilis Cleaves and Inactivates Plasminogen Activator Inhibitor Type 1". *Journal of Biological Chemistry* 276.27 (2001): 24690-24696.

4. Tai M-W and Sweet BV. "Nattokinase for prevention of thrombosis". *American Journal of Health-System Pharmacy* 63.12 (2006): 1121-1123.

5. Sumi H., *et al*. "A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto a typical and popular soybean food in the Japanese diet". *Experientia* 43.10 (1987): 1110-1111.

6. Fujita M., *et al*. "Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan". *Biochemical and Biophysical Research Communications* 197.3 (1993): 1340-1347.

7. Xu J., *et al*. "Thrombolytic effects in vivo of nattokinase in a carrageenan-induced rat model of thrombosis". *Acta Haematologica* 132.2 (2014): 247-253.

8. Hsia C-H., *et al*. "Nattokinase decreases plasma levels of fibrinogen, factor VII, and factor VIII in human subjects". *Nutrition Research* 29.3 (2009): 190-196.

9. Jang J-Y., *et al*. "Nattokinase improves blood flow by inhibiting platelet aggregation and thrombus formation". *Laboratory Animal Research* 29.4 (2013): 221-225.

10. Ero ML BH. "Coagulation profile of nattokinase. Study report of Machaon Diagnostics (Oakland, CA. USA)". In: Data on file, Japan Bio Science Laboratory (2008).

11. Fujita M., *et al*. "Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat". *Biological and Pharmaceutical Bulletin* 18.10 (1995): 1387-1391.

12. Pais E., *et al*. "Effects of nattokinase, a pro-fibrinolytic enzyme, on red blood cell aggregation and whole blood viscosity". *Clinical Hemorheology and Microcirculation* 35.1-2 (2006): 139-142.

13. Sumi H., *et al*. "Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinases". *Acta Haematologica* 84.3 (1990): 139-143.

14. Takaoka S. "Function of Nattokinase (NSK) Excluding Thrombolytic Prevention". Unpublished data. St. Marianna University School of Medicine Intractable Disease Treatment Research Center (2003).

15. Takaoka ST., *et al*. "Effects of Nattokinase on Spontaneous Platelet Aggregation". Paper presented at: The 124th Annual Meeting of the Pharmaceutical Society of Japan (2004).

16. Kurosawa Y., *et al*. "A single-dose of oral nattokinase potentiates thrombolysis and anti-coagulation profiles". *Scientific Reports* 5 (2015): 11601.

17. Yoo HJ., *et al*. "The effects of nattokinase supplementation on collagen–epinephrine closure time, prothrombin time and activated partial thromboplastin time in nondiabetic and hypercholesterolemic subjects". *Food and Function* 10.5 (2019): 2888-2893.

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