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Review

NATTOKINASE – USES AND BENEFITS

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ABSTRACT

Nattokinase has been one of the most widely discussed and researched extracellular enzymes since it was first introduced in 2005. Nattokinase belongs to the subtilisin family and is a proteolytic enzyme (serine protease) with a powerful fibrinolytic effect. Nattokinase is purified and extracted from fermented soybean seeds under the effect of the Bacillus subtilis (Natto) bacteria. Its main natural source is the fermented vegetable cheese called natto, which is a traditional Japanese food consumed in Japan for more than 2000 years (1).

All over the world, natto is regarded as a fibrinolytic miracle food. The enzyme discovery became a fact thanks to the Japanese scientist Hiroyuki Sumi, a researcher at the Medical University in Chicago, who in 1980 after testing more than 173 natural foods as possible thrombolytic agents(2), discovered that natto possesses the ability to break down artificial fibrin in vitro. Later in 1987, Sumi and his team introduced the new fibrinolytic enzyme, extracted from natto, and named it nattokinase (NK), known also as subtilisin NAT. The major interest in the enzyme is namely because of its direct fibrinolytic activity, provided that it remains stable in the gastrointestinal tract after oral administration. This determines it as a highly valuable, safe and easy-to-use nutraceutical with a wide area of medical applications for the treatment of thrombotic, neurological and dyslipidemia conditions, arterial hypertension, diabetes mellitus, atherosclerosis, hemorrhoids, endometriosis, uterine fibroids, muscle spasms, infertility in reproductive medicine and obstetrics.

Key words: nattokinase, fibrinolytic, antithrombotic, anticoagulant, antiaggregant, antiatherosclerotic, antihypertensive, neuroprotective effect, obstetrics, pregnancy.

Origin and Biological Characteristics

Nattokinase is obtained from fermented cooked soybean seeds in the presence of the Bacillus subtilis (Natto) bacteria at a temperature of up to +40°C. The specific ingredients produced by the Bacillus Natto bacteria, are polyglutamic acid, various polyamino acids and nattokinase. Soybeans are an additional source of various vitamins, minerals and proteins, predominatly the B group vitamins, vitamin E, vitamin K2, lecithin, calcium, proteins, etc. The daily consumption of natto explains the longevity of the Japanese.

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As mentioned above in the introduction, NK was discovered in 1980 and introduced and registered in 1987. However, it was not until 2003 that the certification of the extract specification by the Japanese Health and Nutrition Food Association (JBSL) took place. Then, in 2006 it was patented and JBSL became the patent holder. Nattokinase was approved by the U.S. Food and Drug Administration in 2005, and in 2006 – also in Europe (3, 4).

Pharmacokinetic studies have shown that early serum bioavailability is registered around 4 hours post-dose, and the peak serum concentration is reached within 8-9 hours (+/- 3 hours) after oral administration of NK(5). Various clinical studies on animals and human volunteers have shown that NK has a positive overall effect on the

cardiovascular system by lysing blood clots, direct hydrolysis of fibrin and plasmin substrate, converts endogenous prourokinase to urokinase, increases the activity of tissue plasminogen activator (t -PA) and increases the degradation processes of plasminogen activator inhibitor (PAI) (6).

NK activates fibrinolytic but also anticoagulant pathways directly and indirectly. Activation of the fibrinolytic system takes place with the participation of PAI-1 and t-PA. The tissue plasminogen activator, in turn, converts plasminogen to plasmin, and this results in the conversion of fibrinogen to fibrin.

It is there that NK exerts its effect by reducing the concentration of PAI-1 and increasing the activity of tPA, as a result of which the synthesis of plasmin is accelerated and fibrinogen is slowed down by increasing the levels of fibrin degradation products.

On the other hand, NK initiates enzymes which convert prourokinase to urokinase, which in turn further accelerates the process of fibrinolysis. Contrary to this, many articles and studies assume that NK does not affect the conversion of prothrombin to thrombin and slightly influences coagulation in contrast to other fibrinolytics. Natto also directly affects the intrinsic coagulation system by decreasing the antithrombin III activity, which is demonstrated by the increased serum levels of fibrin degradation products and D-dimers (7).

The effect of NK on fibrinolysis and FDP was studied in healthy volunteers who were taking nattokinase for 4 days. The serum FDP concentration was measured before the nattokinase intake and every second day thereafter. A significant increase in FDP was observed as early as the 4th hour, which lasted until the 6th-8th hour, then the concentration of FDP started to decrease gradually. The characteristics of the concentration curve were preserved over the following days, which are a reflection of the continuing degradation of fibrin and confirms the natto biological action permanence (8).

Other studies, in turn, show that there are statistically significant changes in blood viscosity after NK administration. A comparative study among patients taking NK for 3 weeks showed prolonged euglobulin time and a significant drop in the blood viscosity, results which are attributed to increased fibrinolysis associated with the NK administration (9, 10).

The positive effect of NK on erythrocyte aggregation has been proven. It mainly depends on fibrinogen. By reducing fibrinogen, NK reduces erythrocyte aggregation and improves the blood flow. The biological effect of NK on erythrocytes is dose-dependent. The biologically active concentration of NK is reached with 2000 FU orally administered (11).

A number of studies on the NK mechanism of action prove that it actively leads to reducing the blood pressure levels; it prevents blood hyperviscosity and improves blood circulation in the body. The thrombolytic activity of the enzyme prevents thrombus formation, both directly by lysing an existing thrombus and indirectly by increasing plasmin and urokinase levels through direct destruction of plasminogen activator inhibitor (PAI). Nattokinase has a proven plasmin-like biocharacteristic which lyses fibrin directly and indirectly via three different pathways (12):

1. NK lyses fibrin directly;
2. NK increases plasmin levels by increasing prourokinase levels;
3. NK increases the levels of t-PA (tissue plasminogen activator), which acts like urokinase and plasmin.

For the time being, these effects of NK have a proven applicability and relevance in angiology, neurology, cardiology and obstetrics.

Applicability and Benefits

Fibrinolytic and Antithrombotic Effect of NK

Thrombolytic and fibrinolytic therapy is widely used in conditions such as myocardial infarction, cerebral infarction, and pulmonary thromboembolism. Fibrinolytic therapy significantly reduces mortality, as a result of which it has been used widely in medical practice in recent years (13). The most commonly used thrombolytic agents such as plasminogen activators, streptokinase and anisoylated plasminogen streptokinase activator complex,

unfortunately, are used strictly specifically under certain conditions and have a short half-life and often adverse side effects leading to uncontrollable acceleration of fibrinolysis and subsequent hemorrhages which are difficult to control (14). In order to eliminate the adverse effects of artificial thrombolytics, a large number of tests and studies have been conducted on the NK action in the treatment of these diseases. One of the major-scale studies has been conducted by Fujita and his team (15)**.** They have investigated the effect of NK on a common carotid artery thrombosis model in rats and have found out that NK is 4 times more effective than plasmin in thrombus break-down. Estimations show that a dose of 2836 FU of NK lyses up to 88% of the thrombi within 6 hours, and in addition to that, NK also exhibits a prophylactic antithrombotic effect in vivo (16).

Nowadays, it is known that NK degrades fibrin strands directly in thrombi by increasing the synthesis of tissue plasminogen activator (tPA) and suppressing the synthesis of plasminogen activator inhibitor (PAI) (17-19). It has been demonstrated in vivo and in vitro models that NK cleaves PAI molecules (20), and leads to its degradation, and this in turn leads to an increase in tPA levels, which facilitates the higher plasmin synthesis. NK also initiates an enzymatic cascade in which prourokinase is converted to urokinase, which in turn further accelerates the fibrinolysis process.

Studies in human volunteers have shown that oral administration of NK results in a gradual acceleration of plasma fibrinolytic activity which is demonstrated by measuring the ELT (euglobulin lysis time) and tPA levels (21). After administration of natto bacillus (100 mg/kg) to healthy adult volunteers, ELT was reduced and tPA activity was significantly increased ($P < .05$) (22). In a self-controlled clinical study conducted by Hsia et al (23), they found out that after administration for 2 months, the levels of fibrinogen, factor VII and factor VIII were significantly reduced, suggesting a promising cardiovascular benefit associated with the NK administration. Even after a single dose of 2000 FU oral NK, the levels of fibrin degradation products in the blood were significantly increased 4 hours after NK administration ($P < .05$), confirming its effectiveness with regard to thrombolysis and anticoagulation(24).

Anticoagulant and Antiaggregant (Antiplatelet) Effect of NK

In today's practice, the mass intake of low doses of aspirin (85-100 mg/day) as an anticoagulant is very common for the prevention of myocardial infarction, cerebral infarction and atherothrombotic disorders.

Aspirin exerts its effect by inhibiting cyclooxygenase (COX) and subsequently reducing the synthesis of thromboxane A2 (TXA2) in platelets (25). The use of aspirin in the long run, however, has an adverse effect on the gastrointestinal tract (GIT) and results in bleeding in the GIT manifested as abdominal pain, hematemesis and melena. In connection with this problem, some studies prove that NK demonstrates excellent antiplatelet aggregation and antithrombotic activity in in vitro and in vivo models by directly inhibiting the production of thromboxane B2 formation in collagen-activated platelets (10). NK demonstrates a suppressive effect on platelet aggregation induced by adenosine 5'diphosphate and collagen. In addition to that, the positive effects on blood have been found out by reducing the red blood cells aggregation and the blood viscosity (11). These and many other data and studies on a global scale show that NK is a suitable candidate for an antiplatelet and anticoagulant therapy, without the adverse effects of aspirin.

Antiatherosclerotic and Lipid-Lowering Effect of NK

NK is a drug which has a very promising effect on atherosclerosis and reduces the level of lipids in the blood. A number of studies on animals, after oral supplementation with nattokinase, have shown suppression of intima-media thickening in the blood vessels of rats in comparison to the control group of animals(5,9). This intima-media thickness suppression is namely a result of the thrombolytic activity of NK. The difference between the control group and the natto- or NK-fed group was demonstrated for a period of 3 weeks after vascular endothelial damage in rats by suppressing the endothelial thickening.

Chang(26) et al. reckon in their study that NK suppresses the intima media thickening by exerting a synergistic effect, which is due to the antioxidant and antiapoptotic properties of NK. From a different perspective, another large-scale study showed that NK prevents atherosclerosis by exerting its direct antioxidant effect leading to the reduction of lipid peroxidation and improvement of lipid metabolism by inhibiting oxidation of the low-density lipoproteins (LDL) (27).

A large-scale study involving human (28) volunteers taking NK on a daily basis for 26 weeks showed promising results in suppressing the progression of atherosclerosis in patients with atherosclerotic plaques. There was a significant reduction in the carotid artery intima-media thickness (CCA-IMT) and the size of the atherosclerotic plaque in comparison with the pre-treatment baseline parameters.

Figures indicate that the size of atherosclerotic plaques and CCA-IMT decreased from $0.25 \pm 0.12\ cm^2$ to $0.16 \pm 0.10\ cm^2$ and from 1.13 ± 0.12 mm to 1.01 ± 0.11 mm, respectively. mm. This was compared with a group of patients on a concomitant statin therapy (Simvastatin 20 mg). As the results reveal, the decrease in the NK-therapy group is more significant ($P < .01$) than that in the Simvastatin-therapy group. These data suggest that NK is a better alternative to statins, which are often used as a drug to reduce atherosclerosis (28).

Using NK or natto extract containing NK, studies conducted by different (29-34) laboratories confirm that NK has a hypolipidemic effect and may significantly reduce the high levels of serum triglycerides, total cholesterol, and LDL cholesterol (LDL) in animal models. One of the large-scale studies finds out that in hyperlipidemic patients, NK treatment (*26 weeks* at 6500 FU) reduces total cholesterol, LDL and triglycerides(35). In addition to this, NK increases the levels of high-density lipoproteins (HDL), known as good cholesterol.

Antihypertensive Effect of NK

The use of NK has a positive effect on the cardiovascular system as its intake lowers blood pressure levels. In in vivo but also in vitro studies, natto has been found to contain a relatively strong inhibitory effect on angiotensin-converting enzyme (ACE), which has a key role in the conversion of angiotensin I to angiotensin II, in the renin-angiotensin-aldosterone system (36).

This was proved true in 2008 by Kim et al.(37).They conducted the first randomized, double-blind, placebo-controlled trial for the effect of NK on blood pressure in patients with prehypertension and NYHA class I hypertension. They administered NK (2000 FU for 8 weeks) to 86 volunteers aged between 20-80 years and registered a reduction in the systolic and diastolic blood pressure, i.e. -5.55 and -2.84 mmHg, respectively, $P< 05$(37).

The results suggest that NK may play a role in the prevention and treatment of hypertension.

Another large-scale comparable study on the antihypertensive effect of NK and its application was conducted by Jensen et al. (38). They administered NK at a dose of 100 mg/day for 8 weeks to 79 volunteers diagnosed with hypertension, considering the interesting fact that in men arterial pressure is impacted to a larger extent than in women (38).

Considering some significant adverse effects associated with long-term antihypertensive therapy with antihypertensive drugs, NK seems like a promising alternative for the prevention and treatment of hypertension.

Neuroprotective Effect of NK

The neuroprotective effect of NK involves a number of factors and is associated mainly with its antithrombotic activity. One of the earliest studies shows that NK plays a role in the prevention of ischemic brain stroke and exerts a neuroprotective effect in the patients taking it(39). These effects have been demonstrated for the first time in photothrombotic stroke models in mice (40), and subsequently, two large-scale studies have proven its role and mechanism of action. Ahn and colleagues present (40) data that the neuroprotective effect of NK is seen in the improvement of the blood flow, inhibition of platelet aggregation and facilitating neovascularization in cerebral ischemic tissue. Another large-scale study with evidence for the neuroprotective effect of NK is the one conducted by Ji et al. (41), in which they describe:

1. Antithrombotic activity, which takes place by increasing the levels of cyclic adenosine; monophosphate (camp), reduces the passage of calcium through the vascular endothelial cells and its release from the calcium stores;
2. Antiapoptotic effect by activating the JAK1/STAT1 pathway;
3. Relaxes vascular smooth muscle cells by means of synthesis and release of nitric oxide (NO) and the protection of endothelial cells through its fibrinolytic and thrombotic properties.

Another significant and proven effect of NK is the ability to degrade amyloid at neutral pH and normal body temperature, which is used in the treatment of disorders associated with amyloid accumulation such as Alzheimer's disease (AD) (42).

This effect has been proven by s both in vivo and in vitro model studies. The formed amyloid fibrils are protein aggregates with a lamellar cross-beta structure (amyloid-β). Amyloid-β plays a role in the activation of acetylcholinesterase (AchE) in the brain and the reduction of the brain-derived neurotrophic factor (BDNF). NK as serine protease participates in the degradation of amyloid fibrils, which results in the inhibition of the AchE activity and restoration of the BDNF levels (43).

Ahmed and colleagues demonstrate that NK at a dose of 360 FU/kg significantly reduces AchE but also TGF-β, IL-6 and p53 levels, accompanied by a significant increase in the Bcl-2 levels in comparison to an untreated AD control group (44).

Effect of NK in Obstetrics

In normal pregnancy, the changes in the blood leading to hypercoagulation have been proven for decades and explained from both a physiological and a pathophysiological point of view. Pregnancy results in changes in the coagulation and fibrinolytic systems and increases significantly the risk of thromboembolism.

A normal pregnancy results in an increase in most of the coagulation factors: factors VII, VIII, X, XII and fibrinogen, while protein C and protein S in the group of the coagulation inhibitors usually decrease (45). Of significant importance is the study of plasminogen activator inhibitor (PAI), which shows a tendency to increase during pregnancy. The prothrombin and factor V values remain unchanged. PAI-1 and PAI-2 (produced by the placenta) which inhibit fibrinolysis, are elevated, especially in the third trimester (46).

The tendency for developing thrombosis during pregnancy is significant, especially in the case of congenital thrombotic disorders with mutations in some of the genes of methylenetetrahydrofolate reductase (MTHFR), plasminogen activator inhibitor (PAI – 4G/4G, 4G/5G, 5G/5G) and the Leiden factor V, and less often deficiency of antithrombin, protein C and S.

On the basis of the presented significant effects of NK as an antithrombotic, antifibrinolytic, antiaggregant, anticoagulant, and arterial blood pressure-reducing medication, it is widely used in the medical practice for pregnant women, although there is no targeted research among pregnant women at high thrombotic risk. NK is effective when administered orally and its safety for the fetus is proven by the millennia-old use of natto cheese as a food in Japan.

CONCLUSION

Nattokinase demonstrates several key benefits in the treatment of thrombosis, hypertension, atherosclerosis, hyperlipidemia, platelet aggregation, and neuroprotection in patients. These numerous benefits make the role of NK unique in the prevention and treatment of a number of socially significant diseases. It has a safe profile, low cost, fast and simple manufacturing process, it is suitable for oral administration and has a long in vivo half-life. This makes it a good alternative to the traditional antithrombotic, antihypertensive, antihyperlipidemic and neuroprotective medications.

However, there are several challenges which have to be overcome so that NK becomes a common drug, i.e. the accurate pharmacokinetics of absorption and metabolism, and further studies to explain drug interactions with other classes of drugs.

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