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Review Article

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NATTOKINASE ENZYME; AN EVALUATION OF ITS CELLULAR AND POTENTIAL THERAPEUTIC ACTIONS

Dr. Manoj G. Tyagi*

Department of Pharmacology, Christian Medical College, Vellore 632002, Tamilnadu, India.

***Correspondence for Author: Dr. Manoj G. Tyagi**
Department of Pharmacology, Christian Medical College, Vellore 632002, Tamilnadu.

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ABSTRACT

Fibrinolytic enzymes have been derived from different microorganisms, including the genus *bacillus* which occurs in traditional fermented foods. Nattokinase (NK) is an enzyme contained in the sticky component of natto, a cheese-like food made of soybeans fermented with *Bacillus subtilis*. Nattokinase has been shown to have fibrinolytic and has effects on the coagulation system which are beneficial for cardiovascular ailments. Recent research has shown that this enzyme may also have neuroprotective and also effects on the smooth muscle acvity with potential benefits for the Alzheimer's disease, hypretension and vitreoretinal disorders. This review article focusses on the cellular and pharmacological actions of Nattokinase as well mentioning some experimental effects on gastrointestinal system.

KEYWORDS: Nattokinase, fibrin, cardiovascular, smooth muscle, enzyme.

INTRODUCTION

Nattokinase enzyme was discovered in Natto, a fermented cheese-like food that has been used in Japan for over 1000 year.[1] Nattokinase is a potent fibrinolytic enzyme produced by a fermentation process by adding *Bacillus* natto, a benefical bacteria, to boiled soybeans, it is only the 'natto' preparation that contains the specific nattokinase.[2] Nattokinase is particularly potent treatment, and it enhances the body's natural ability to fight blood clots in several different ways; because it so closely resembles plasmin, it dissolves fibrin directly. In addition, it also enhances the body's production of both plasmin and other clot-dissolving agents, including urokinase (endogenous).[3] An *in vivo* study of the duodenal absorption of nattokinase in rats showed intact absorption of the enzyme. This was based upon the subsequent degradation of plasma fibrinogen clearly demonstrating transport of nattokinase across the intestinal tract. The action of nattokinase on the cleavage of fibrinogen in the plasma was remarkably prolonged being present in plasma samples drawn 3 to 5 hours after administration of the enzyme.[4,5] It digests fibrin both directly and indirectly. Indirectly, it activates pro-urokinase and tissue plasminogen activator (t-PA), supporting the fibrinolytic activity of plasmin. These combined actions promote healthy platelet function, contribute to the regular healthy function of the heart and cardiovascular system by maintaining proper blood flow, thinning the blood and preventing blood clots.[6,7] There are also recent reports about the beneficial effects of nattokinase on cerebral ischemia.[8] Recent studies have suggested that Nattokinase produced from mutant strain P.aeruginosa CMSS UV 60 showed enhanced production and blood clot lysis than compared with Bacillus subtilis.[9,10] This article reviews the cellular and current therapeutic status of Nattokinase enzyme.

Nattokinase and potential therapeutic roles: Cardiovascular studies conducted in experimental animals have depicted that the angiograms show the blood clots in the dogs that received nattokinase had completely dissolved within 5 hours of treatment, and that normal blood circulation had been restored. Blood clots in the dogs who received the placebo displayed no signs of dissolving 18 hours after the treatment.

In another study, researchers from Biotechnology Research Laboratories and JCR Pharmaceuticals Co. of Kobe, Japan, tested Nattokinase's ability to dissolve a blood clot in the carotid arteries of rats. Animals treated with Nattokinase regained 62 percent of blood flow, whereas those treated with plasmin regained just 15.8 percent of blood flow.[11]

In a laboratory study conducted on blood vessels, endothelial damage was induced in the femoral arteries of rats that had been given Nattokinase. In normal circumstances, a thickening of the artery walls and blood clotting would occur, but they were both suppressed due to the Nattokinase's fibrinolytic activity. There is extensive research that explains exactly how nattokinase exerts its benefits throughout the circulatory system. The use of animal studies is most convincing, because this research closely mimics how nattokinase works in the

human circulatory system. The study concluded that the supplementation of nattokinase reduced blood pressure in human subjects that initially had high blood pressure ranging from 130 to 159 mmHg.[7] While this study questioned the ability of nattokinase to decrease arterial thickening, the researchers did report that nattokinase is capable of lowering both systolic and diastolic blood pressure. The researchers suggest that nattokinase works on the blood pressure pathway controlled by the kidneys, inhibiting the activity of certain chemicals that are responsible for raising blood pressure. A 2009 study out of Taiwan studies the ways in which nattokinase improves circulation. Researchers found that nattokinase benefits the circulatory system through two separate mechanisms: first, through directly breaking down blood clots, and second, by converting precursors of fibrin into inactive forms. This is a significant benefit, because fibrin can be a major risk for developing blood clots and arterial blockages. Compounds in natto have also been identified as angiotensin-converting enzyme inhibitors, which lower blood pressure.[12] A study conducted by the present author showed the potential beneficial effect of nattokinase and endothelin receptor antagonist for blood pressure and cardiac failure.[13] Nattokinase has also been shown to induce vitreolysis for retinopathy and other macular diseases.[14] Therefore, regarding the PVD-inducing mechanism, it is postulated that nattokinase may have two major effects: one is the direct effect of liquefying the vitreous gel by its proteolytic activity and the other is the indirect effect of increasing the plasmin activity that induces the vitreoretinal dehiscence. The following is a list of conditions likely to be ameliorated with use of nattokinase:

1) *Atherosclerosis 2) Coronary artery disease*—via heart attack prevention, morbidity, and recurrence reduction
3) *Hypertension* 4) *Peripheral vascular disease*—arterial
atherosclerosis, venous thrombi 5) *Strokes*—prevention, and morbidity and
reduction recurrence 6) *Thrombus formation*—including, venous
clots, arterial-wall thrombi with atherosclerosis, atrial-chamber thrombi
(as in occurs in chronic atrial fibrillation), hemorrhoids, eye thrombosis
(vena centralia retinae acresia), and senile dementia associated with cerebral
thrombi formation.[15]

Nattokinase activity measurement: The enzyme activity was quantified by cleavage of the synthetic substrate N-succinyl-Ala-Ala-Pro--Phe-p-nitroanilide, as determined by absorbance of product (p-nitroaniline) at 410 nm. The reaction mixture containing 0.5 mL of 1 mM synthetic substrate, 0.6 mL of PBS buffter, and 0.5 mL of the enzyme solution was incubated at 37 °C for 10 min. The reaction was then stopped by adding 0.5 mL of 0.2 M acetic acid. The absorbance of released p-nitroaniline can be measured at 410 nm using a UV spectrophotometer (UV2500, LaboMed Inc., Culver city, CA, USA). One unit of amidolytic activity was expressed as nmol of p-nitroaniline released due to substrate hydrolysis/min/mL by the enzyme.[16,17]

Nattokinase and postulated signal transduction mechanism: Nattokinase also significantly increases the cAMP level, and activates the signal passage of JAK1/STAT1 in injured part and inhibited remarkably the rise of platelet intracellular Ca^{2+} ($[Ca^{2+}]_i$) in human platelets. Furthermore, Nattokinase (NK) relaxed rat thoracic aortic artery in the dose-dependent manner and in the endothelium dependent manner and its effect could be attenuated by l-NAME. Also, the secretion of t-PA and PAI-1 were reduced stimulated by Adr on Huvecs. These data indicated that the neuroprotective effect of NK was associated with its anti-platelet activity by elevating cAMP level and attenuating the calcium release from calcium stores; with its anti-apoptotic effect through the activation of JAK1/STAT1 pathway; with its relaxing vascular smooth muscle by promoting synthesis and release of Nitric oxide.[8,18] In a recent article it was show that the Nattokinase nanofibers come in contact with blood, the NK is released from the nanofibers to resist platelet adhesion on the nanofiber surface, facilitating the direct capture and isolation of red blood cells (RBCs) from the blood above phase-transition temperature of PNIPAAm. Meanwhile, the captured RBCs are readily released from the nanofibers with temperature stimuli in an undamaged manner. The release efficiency of up to 100% is obtained while maintaining cellular integrity and function. This work presents promising nanofibers to effectively capture non-adherent cells and release for subsequent molecular analysis and diagnosis of single cells.[19]

Isolated goat ileum preparation: A study was conducted on the effects of nattokinase on goat smooth ileum. The results showed that nattokinase displayed relaxation of ileum smooth muscle. The results are depicted in Table 1. The technique used was a modified version. A Goat ileum was procured from local sources. A piece of goat ileum was removed, cleaned and was placed in a petri dish containing Tyrode solution. A thread was attached to the top to serve as a marker. The perfusion fluid in petri dish was aerated and debris inside the lumen was washed gently with pipette. The mesenteric membrane was trimmed for a length of ileum of approximately 2 cm. Two threads were tied to the upper and lower portion of the gut.The thread tied to the lower portion was attached to the hook of the air-delivery tube inside the bottom of thechamber, in a water jacketed organ bath containing 20 ml Tyrode solution (composition in mM: NaCl 136.89, KCl2.68, MgCl2 1.05. CaCl2 1.36, NaH2PO4 0.32, NaHCO3 11.90 and glucose 5.55) and the thread tied to the upper portion of gut was attached to the force displacement transducer. Tissues were mounted under an initial load of 1.0 g and allowed to equilibrate for 30 min. before the addition of

any drug. The experiments were performed at 37 °C and bubbled with a mixture of air produced by a motorized areator. Normal rhythmic motility was recorded on a student's electric kymograph (Bio-Device, Ambala).[20]

Table 1: Effect of Nattokinase on GI motility in goat ileum

Pretreatment	Treatment	Amplitude	% Change	'p'value
Nil	KCl	3.95 ± 0.78	-	
Nil	Ach	3.99 ± 0.81	-	
KCl (60mM)	Nattokinase (20mg)	3.11± 0.57	21.27	P < 0.05
Ach (400nM)	Nattokinase	2.92 ± 0.47	26.82	P <0.05

CONCLUSION

Natto contains a potent fibrinolytic enzyme that they named as nattokinase, and an oral form is now available to consumers worldwide as a supplement without a physician's prescription. This 275 amino acid has similar sequences to other natural endogenous enzymes. Sumi *et al* claimed that it closely resembles plasmin and strongly hydrolyzes fibrin. It was also suggested that nattokinase has been the most potent fibrinolytic enzyme among 200 foods investigated for oral fibrinolytic therapy. Nattokinase has been of considerable interest because of its capacity to digest fibrin in blood vessels. The enzyme is currently considered in pharmaceutical industry as a promising drug for thrombolytic therapy. In addition, these enzymes have significant potential for food fortification and nutraceutical applications, such that their use could effectively prevent cardiovascular disease. In the future, the research will progress into the production of highly purified fibrinolytic enzymes from bacterial sources, it is still the most stable and economic way to obtain protein with fibrinolytic activity by *B. subtilis.*

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