Nattokinase historical sketch on experimental and clinical evidence

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ABSTRACT

Nattokinase (NK) is a protease derived from food used mainly in the Japanese diet that has several properties. The main activity is related to improving fibrinolytic activities. Other activities have been demonstrated in the regulation of blood pressure by the action toward angiotensin proteases and in the antiplatelet activities. NK can be given orally and reaches its maximal concentration after 12 hours. In addition, an antithrombotic activity based on various NK activities has been proposed. First, increased fibrinolytic activity increases thrombus dissolution and/or the formation of atherosclerotic plaques; second, its enhanced antiplatelet action adds to clot dissolution. All activities have been studied in animals and humans in vitro and *in vivo*. Relevant adverse effects of NK therapy have not been described, however clinical experience is restricted to case series and volunteers and is not based on clinical studies, thus clinical trials are required to confirm.

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Introduction

Nattokinase (NK) is a serin protease of vegetal origin, and it can derive from specific food such as natto.[1] In particular, NK may be derived by a specific process of fermentation of soybean by adding specific bacteria such as Bacillus subtilis.[2] It has been reported that NK may exert its proteolytic action in a specific way toward such proteins of the fibrinolytic pathway,[3] and in a non-specific way; it also works toward other circulating proteins interacting mainly with endothelial cells.[4] For these reasons, NK is considered a possible relevant drug that may be involved in the primary or secondary prevention of thrombotic diseases.[5] After the evaluation of the National Science Foundation in the United States of America, the general audience toward NK is increasing, and studies *in vitro* and *in vivo* are increasing too.[6] We here report current pieces of knowledge of NK actions.

Nattokinase origins

NK became known after the identification of bacillus subtilis natto B12 which was isolated after the fermentation of natto. Natto is a traditional food of the Japanese diet.[7] Several historical sketches are available to date the initial origin that introduced natto in the Japanese diet, and is all spanning to the Samurai era. In this third millennium, the globalization of Japanese

foods supported the attention on this special vegetable.[7] NK derived after fermentation of natto and following action of bacillus subtilis B12, in fact, showed several activities on the cardiovascular system, in particular, regarding a specific profibrinolytic action.[8]

Nattokinase properties

The identification of NK was performed by analysing the supernatant of bacillus subtilis B12 derived from the fermentation of natto. NK has been identified as a specific monomer of nearly 30 KDa.[7] It shows a relative thermostability (wang) between 30 and 50°C and alkaline stability with pH between 6 and 8.[8] It can be adsorbed by oral intake, and its serum peak can be planned within 12 hours after the intake.[9]

Its specific action is directed toward the fibrinolytic system acting in multiple ways: increasing the release of endogenous tissue plasminogen activator (tPA), reducing the release of plasminogen activator inhibitor type 1 (PAI-1), and making easy the conversion of endogenous pro-urokinase to urokinase. All these actions can strongly increase the fibrinolytic way so, increasing active plasmin and so the degradation of non-stabilized fibrin.[4,9] Recent studies demonstrated that NK acts mainly as a subtilisin than a specific serin protease,[10] however its pharmacological action is based on the activation of plasminogen in plasmin by a specific cleavage.[9,11] In this way, heparinoids such as heparins or heparan sulfate seem to not be able to increase the protease activity of NK.[11] Furthermore, NK actions as protease may also interest other proteases' systems as those involved in the clotting cascade, platelet aggregation, blood pressure control and atherosclerosis.[10-14] Several hypotheses have been suggested for other interactions between NK and other systems. Regarding blood pressure, several reports testified that NK or its fragments are able to inhibit and/or cleave angiotensin-converting enzyme I or angiotensin II so, giving a better regulation of blood pressure.[10] Clotting activities with a trend to anticoagulation of NK have been testified by cleaving fibrinogen and other clotting factors such as factor VII and factor VIII after oral intake.[12] Antiplatelet actions and improved haemoreological actions have also been reported for NK. Its antiplatelet action has been testified by several studies *in vitro* that underlined a reduced ability of platelet to exposition to collagen, then inducing a reduced release of ADP and thromboxane A2 so, reducing platelet aggregation.[13] Furthermore, as a subtilisin, NK has been shown to have a considerable ability to promote cleavage of beta-amyloid, which has been linked to the advancement of atherosclerosis. This activity could be involved, in fact, in the reduction of atherosclerotic plaques reported in different animal studies, in particular in small vessels. If confirmed, in humans, this additional activity of NK could also be involved in the prevention of several neurological diseases.[15]

Nattokinase studies on animals

The efficacy of NK to perform clot lysis was testified *in vitro,* cleaving cross-linked fibrin in a more effective way of plasmin *in vitro*. Furthermore, *in vitro* studies showed that NK is also able to increase the release of tPA and the cleavage of plasminogen activator inhibitor-1.[2] After, the identification of its action *in vitro*, NK has also been tested in animals.

In animal models, the efficacy of NK was demonstrated using thrombolysis *in vivo* after chemical-induced thrombosis. Restoration of blood flow was obtained, and it was directly proportional to the amount of NK injected in rats.[16] In other rat models, NK was able to carry out thrombolysis in carrageenan-induced thrombosis.[17,18] Furthermore, also in dogs, blood flow was restored with full recanalization after 5 hours of oral NK administration.[19]

Nattokinase sudies on humans

Based on its positive results *in vitro* and in animal models, in particular for thrombus dissolution, NK has also been tested in humans in several studies. Studies on healthy volunteers demonstrated that after oral intake of NK, several haemostatic parameters changed, underling improved fibrinolytic action.[20] NK used in patients who underwent vascular surgery was able to reduce clinical symptoms such as leg pain, and skin pain.[21] Furthermore, in another study *vs.* placebo, NK was also used and testified as effective in preventing DVT after flying.[22] On the other hand, in a study *versus* placebo, the use of NK was able to reduce subclinical atheroscelrosis measured by serial carotid ultrasound every six months as carotid artery intima-media thickness.[23]

Current limitations

The main limitation that can be found on the use of NK for primary or secondary prevention of cardiovascular disease is based on the absence of clinical trials, in particular, phase III clinical studies are lacking for this compound. Furthermore, all available studies in humans testified to the efficacy of NK *vs.* placebo. Reports that we cited and that are actually available are mainly real-life studies, case series or studies on healthy volunteers so, leaving uncertainties on the large use of NK, in particular for long-term treatments. Therefore, the next challenges for NK will regard the possible plan of randomized clinical trials. In this way, several reflections may be done before planning any type of randomized clinical trials, in particular, if they will be focused on the primary or secondary prevention of thrombotic diseases and if they will be

focused on the prevention of atherothrombosis or venous thromboembolism. Moreover, being a product that acts as profibrinolytic as far as anticoagulant, bleedings have been reported as the main complication of the assumption of NK in several reports.[24,25]

Conclusions

Although atherothrombosis and venous thrombosis are well-known diseases, and we have highly effective tools for primary or secondary prevention of these thrombotic diseases, actually they still represent the most common causes of morbidity and mortality in Western countries. Besides improvements in lifestyle and multidrug-tailored prevention, several new data are emerging on the role of endothelial dysfunction and chronic inflammation and thrombotic disorders. The development and the discovery of new targeted drugs that may interact with proteins involved in the relationship between inflammation and thrombosis are new goals for the primary and secondary prevention of thrombotic diseases. For these reasons, the occurrence of drugs that may act with proteases/antiproteases balance may be useful because it may interact with the clotting system, with fibrinolytic pathway besides with complement system and cytokines' network. In this way, NK can interact with reported systems and also with the RAS system.

NK represents one of the most promising drugs that act on proteases\antiproteases balance, nad its affinity seems to be relevant as a profibrinolytic drug. However, its ability to improve functions of the RAS system controlling hypertension and kallikrein system moving actions toward anticoagulation are additional positive effects. Furthermore, also a preventive action toward the reduction of atherosclerotic plaques is intriguing for primary prevention of microvascular diseases such as dementia. As with all drugs that can treat thrombosis, bleedings are the most common side effect of NK based on available reports in humans.

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