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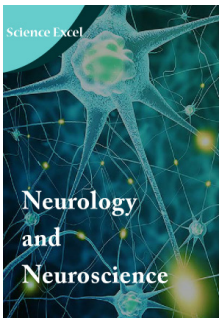
Original Article



Use of Nattokinase in patients with ischemic stroke and transient ischemic attacks

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Abstract

The concept of Nattokinase as an alternative to the standard prevention in acute ischemic cerebrovascular incidents is interesting and promising.

Two groups of patients were considered – ones taking Nattokinase at a dose of 160 mg, 3000 fibrinolytic units of activity for 24 hours (n=96) and a control group, in which the secondary prevention was based on antithrombotic drugs – Acetylsalicylic acid (325/100 mg) and/or Clopidogrel (75 mg) daily (n=95).

At a 6-month follow-up interval, no statistically significant difference was observed in the vascular (brain and cardiac) complications between the two categories monitored. The occurrence of adverse reactions in both groups was also statistically insignificant, in support of the reported literature and own initial data.

Introduction

Antithrombotic drugs (Class I, Level of Evidence A) are used for secondary prevention in patients with cerebral infarction and transient ischemic attacks (TIA) [1]. It is essential to reduce the risk of subsequent events following acute ischemic cerebrovascular events [2].

The antiplatelet action of Acetylsalicylic acid (ASA) does not occur in all patients and between 8% and 45% of people are „aspirin resistant" [3]. Clopidogrel is more effective than self-administered ASA and is globally recommended for secondary prevention of cerebral infarction [4,5]. Between 5% and 30% of patients receiving Clopidogrel show low or lack of efficacy, which is known as the term „clopidogrel resistance" [6,7].

In a study of 1186 patients affected by ischemic stroke that have been monitored within 3 years [8], 432 of them have received ASA as a secondary prevention treatment, and 382 - Clopidogrel. In the group with ASA administration, 32 patients underwent secondary and 4 - third hospital admission. Of these, 25 had a new cerebral infarction and 3 had an intracerebral hematoma. Among the patients undergoing secondary prevention treatment with Clopidogrel there were 33 re-admissions and 3 – for the third time, as 26 were for a new ischemic stroke and 1 for parenchymal intracerebral haemorrhage.

The incidence of recurrent cerebrovascular accidents, including in the self-cited observation demonstrates complete or partial clinical resistance to ASA and/or Clopidogrel (in some cases), regardless of the proven efficiency of these drugs. This directs research interest in the discovery and development of new active molecules and combinations, aiming successful secondary prevention in each patient with cerebral infarction and TIAs in daily practice.

According to numerous publications the enzyme Nattokinase has a neuroprotective mechanism of action (as a result of proteolytic, anti-inflammatory and anti-apoptotic effects) in experimental models of ischemic stroke and slows the development of atherosclerosis [9-11].

Nattokinase is an alkaline protease of 257 amino acid parts with multilayer properties, derived from fermented soy product (Natto), a traditional food in Asia [12,13]. Based on in vitro and in vivo studies, its specific characteristics have been identified - potential fibrinolytic and antithrombotic activity in arterial and venous thrombosis, decrease in blood viscosity and erythrocyte aggregation and a positive effect on controllable risk factors such as arterial hypertension and dyslipidemia [14-16].

In 2012 J-M Wang et al. reported that in animals with experimentally induced focal cerebral ischemia Nattokinase reduced fibrinogen levels and reduced infarction volume by 54-68%. These processes were performed in a cascade-mediated pathway similar to ASA [9,17].

Based on an established model of photothrombotic cerebral infarction in mice Y-J. Ahn et al. [18], confirm the neuroprotective effect of Nattokinase by reducing arterial thrombosis and stroke volume at the 4-th hour of therapy (26.2 ± 6.5 mm^3) compared to controls (42.9 ± 2.5 mm^3). In addition, there is an improvement noted in blood flow in the ischemic area of injury and in the non-ischemic area [9].

We present results for the use of oral Nattokinase as an alternative for secondary prevention in acute ischemic cerebrovascular accidents.

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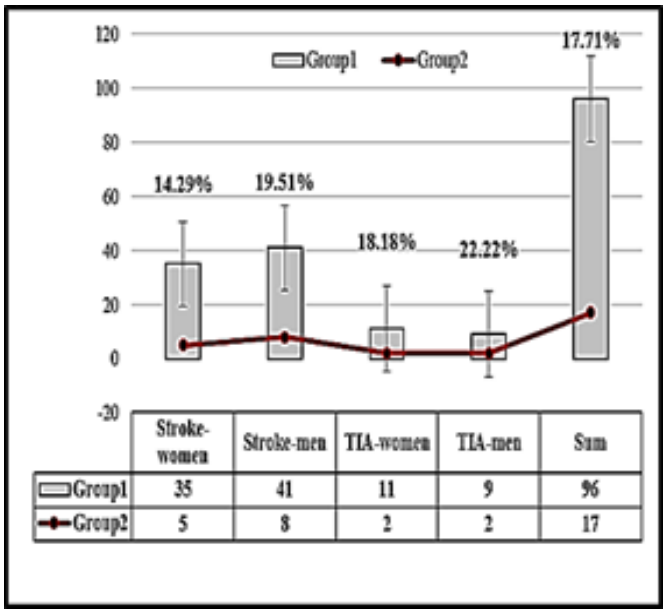

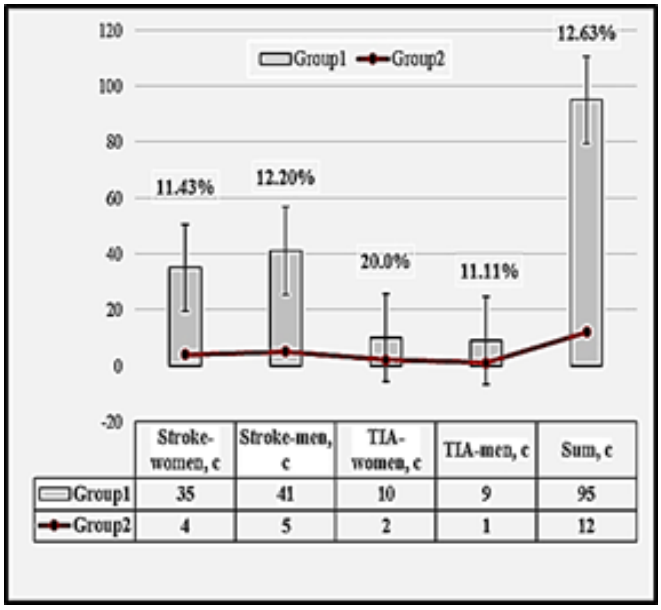

Figure 1. Frequency distribution of accompanying malignant disease in patients with stroke and TIA receiving Nattokinase (1.1) and in controls (1.2).

*With "Group 1" conditionally denoted the studied subjects and with "Group 2" - concomitant oncological comorbidity.

Model and methods

During the period 01.2017-03.2019 96 patients with ischemic stroke and TIA who underwent treatment at the Clinic of Neurology, University First MHAT - Sofia started taking Nattokinase at a dose of 160 mg, 3000 fibrinolytic units for 24 hours once daily. A standard diagnostic and therapeutic panel according to the national Guidelines and Recommendations were performed as well as follow-up meetings. Patients and caregivers have been informed that this is a product with the official registration as a food supplement (Nataspin H®), and an informed consent for clinical follow-up was obtained for each participant and/or companion.

For the purpose of the comparative analysis, the trial included a control group of 95 other patients with stroke and TIA in which secondary prevention was based on antithrombotic drugs - ASA (325/100mg) and/or Clopidogrel (75mg) daily. The characteristics of the patients monitored (taking Nattokinase and control group) are presented in Table 1.

Clinical characteristics	Patients receiving Nattokinase, n=96	Control group, n=95
Age ± SD	74.6 ± 7.5	71.9 ± 8.2
Sex Female Male	 46 (47.9%) 50 (52.1%)	 45 (47.4%) 50 (52.6%)
With ischemic stroke Female Males	76 (79.2%) 35 (36.5%) 41 (42.7%)	76 (80.0%) 35 (36.8%) 41 (43.2%)
With TIA Female Males	20 (20.8%) 11 (11.4%) 9 (9.4%)	19 (20.0%) 10 (10.5%) 9 (9.5%)

Table 1. Baseline characteristics of the study participants.

The main indications for selection are: concomitant gastrointestinal disorders associated with a moderate and high risk of bleeding (62.5%), history of cerebral hemorrhage (6.3%), rare episodes of atrial fibrillation/flutter (12.5%), individual intolerance or unwillingness for treatment with antithrombotic drugs (18.7%). In 17.7% of patients there was comorbidity with cancer compared to 12.6% of the control group (Figure 1).

Results and discussion

The Trial of ORG 10172 in Acute Stroke Treatment (TOAST) is conditionally used in this statement, according to which ischemic cerebrovascular accidents can be divided in 5 groups [19] with atherosclerosis of large blood vessels – aorta, carotid and vertebral arteries cardioembolic with engaging of the small blood vessels other subtypes with determined etiology (due to migraine, vasculitis, vasculopathies, venous thrombosis) cryptogenic - with unknown etiology.

According to TOAST in the group of patients with cerebral infarction and TIA, who were treated with Nattokinase (n=96) the main etiologic factors are athero- and arteriolosclerosis – in 87.5% of all cases, and only 12.5% of them can be considered as caused by cardiac sources of embolism. In the control group (n=95) the leading causes are large-artery atherosclerosis and small vessel occlusion - in 92.6% versus 7.4% of cases with possible cardioembolic etiology

The analysis includes patients with ischemic strokes and TIA (Nattokinase group and a control group) with predominantly thrombotic origin.

The thesis of our research paper is the application of Nataspin H® as a mean of secondary prevention in two directions – effectiveness and safety. Cerebrovascular accidents with mixed or cryptogenic etiology are not included in the representative samples.

Patients' response was monitored at 1, 3 and 6-month intervals. In both groups around or under 8.0% of subjects did not attend a scheduled follow-up visit.

In patients with Nattokinase for a period of 30 days, one case of TIA with criteria for previous high-risk profile, and two cases of ischemic stroke were identified, one of them with severe neurological deficit.

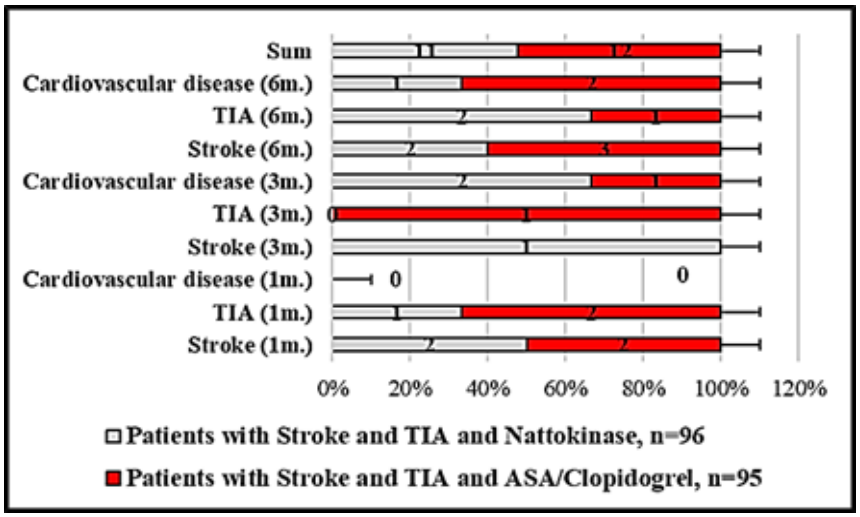

Figure 2. Vascular (cerebral and cardiac) incidents occurred within the 1, 3- and 6-month follow-up period between the two groups.

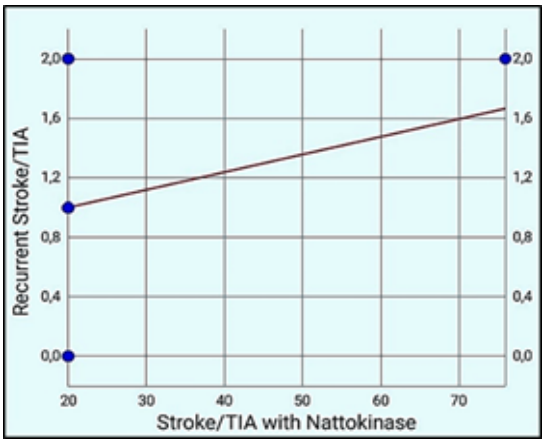

Figure 3.1

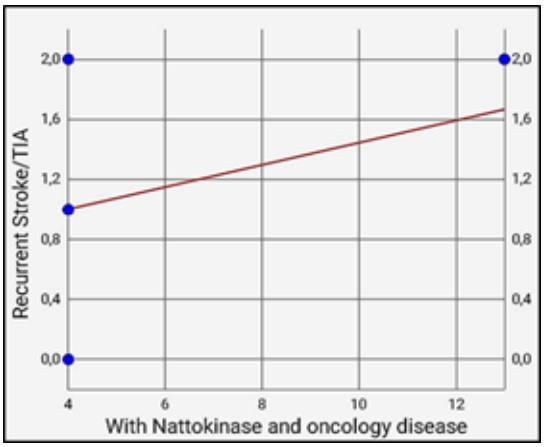

Figure 3.2

Figure 3. At 1, 3 and 6 month intervals - recurrent Stroke/TIA = 0.0119 (Stroke/TIA with Nattokinase) + 0.7619. R = 0.4472, R^2 = 0.2, P = 0.3739 (Figure 3.1) and recurrent Stroke/TIA = 0.0741 (Stroke/TIA with cancer comorbidity and Nattokinase) + 0.7037. R = 0.4472, R^2 = 0.2, P = 0.3739 (Figure 3.2).

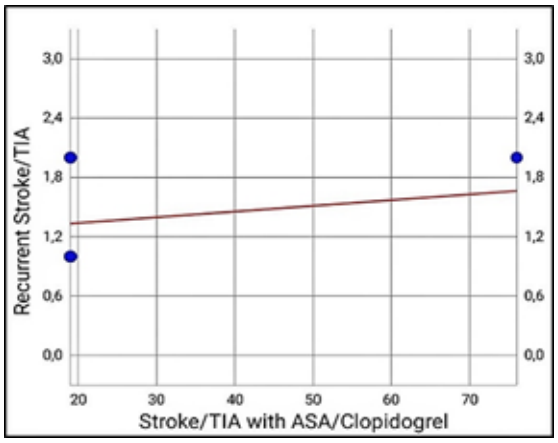

Figure 4.1

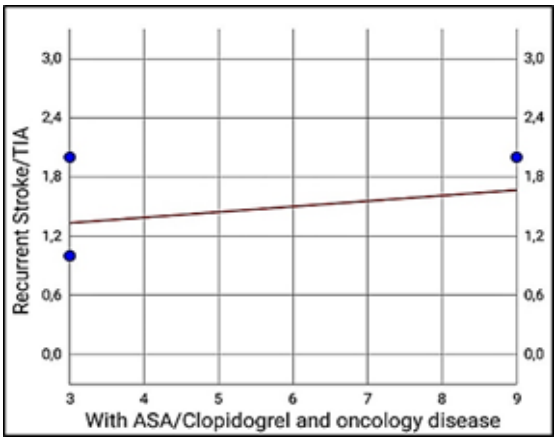

Figure 4.2

Figure 4. At 1, 3 and 6 month intervals - recurrent Stroke/TIA = 0.0058 (Stroke/TIA with ASA/Clopidogrel) + 1.2222. R = 0.1741, R^2 = 0.0303, P = 0.7415 (Figure 4.1) and recurrent Stroke/TIA = 0.0556 (Stroke/TIA with cancer comorbidity and ASA/Clopidogrel) + 1.1667. R = 0.1741, R^2 = 0.0303, P = 0.7415 (Figure 4.2).

	Recurrent Stroke/TIA	Stroke/TIA with Nattokinase	Stroke/TIA with oncological comorbidity and Nattokinase
Recurrent Stroke/TIA	-----	0.0011*	0.7718
Stroke/TIA with Nattokinase	0.0011*	-----	0.0045*
Stroke/TIA with oncological comorbidity and Nattokinase	0.7718	0.0045*	-----

Table 2. Multiple comparison test in patients with stroke and TIA receiving Nattokinase.

	Recurrent Stroke/TIA	Stroke/TIA with Nattokinase	Stroke/TIA with oncological comorbidity and Nattokinase
Mean	1.3333	48	8.5
Standard deviation (SD)	0.8165	30.6725	4.9295
Standard error of the mean (SEM)	0.3333	12.522	2.0125
95 % CI of mean	(0.48) – (2.19)	(15.81) – (80.19)	(3.33) – (13.67)

Table 3. Intra-group analysis and statistical parameters of the separate categories.

	Recurrent Stroke/TIA	Stroke/TIA with ASA/Clopidogrel	Stroke/TIA with oncological comorbidity and ASA/ Clopidogrel
Recurrent Stroke/TIA	-----	0.0014*	0.9038
Stroke/TIA with ASA/Clopidogrel	0.0014*	-----	0.0034*
Stroke/TIA with oncological comorbidity and ASA/ Clopidogrel	0.9038	0.0034*	-----

Table 4. Multiple comparison test in patients with stroke and TIA receiving ASA/Clopidogrel.

	Recurrent Stroke/TIA	Stroke/TIA with ASA/Clopidogrel	Stroke/TIA with oncological comorbidity and ASA/ Clopidogrel
Mean	1.5	47.5	6
Standard deviation (SD)	1.0488	31.2202	3.2863
Standard error of the mean (SEM)	0.4282	12.7456	1.3416
95 % CI of mean	(0.4) – (2.6)	(14.74) – (80.26)	(2.55) – (9.45)

Table 5. Intra-group analysis and statistical parameters of the separate categories.

Within the 3 and 6-month monitoring, there were three recurrent ischemic strokes, two TIAs, and cardiovascular complications were observed in three of the subjects.

In the control group with ASA/Clopidogrel intake within 30 days four patients had cerebral infarctions and TIA. Within 3 and 6 months of follow-up there were two cases of TIA, three of stroke and three of cardiovascular complications. The distribution of cardiac and cerebral complications is presented in Figure 2.

When comparing subsequent cerebrovascular accidents, no statistically significant difference was found in the two groups (p=0.18, calc. t=1.73, crit. t=3.18). The incidence of cardiovascular problems is identical. When comparing the total number of vascular complications again, no statistically significant difference was found (p=0.51, calc. t=0.69, crit. t=2.26).

According to the correlation coefficient R, in the group of patients taking Nattokinase, a positive and upward relationship was observed between the individual values (Figure 3). R^2 100% is 20% of the power of influence of the dependent variable, which is an unsignificant result and requires the inclusion of other independent variables (k2=0.5528). In the control group, a positive and upward dependence

can be commented on according to the same coefficient (Figure 4). R^2 100% is only 3.0% of the power of influence of the dependent variable (k2=0.9697) [20-22].

Using linear regression analysis, it was found that the null hypothesis H0 was not rejected (by standard criterion $p<0.05$) or the regression model did not explain the variability in the dependent magnitude of the number of subsequent cerebrovascular events (Figures 3 and 4). Between the separate groups: recurrent stroke/TIA for 1, 3 and 6 months, total number of cerebral infarctions and TIA with Nattokinase and stroke/TIA with oncological comorbidity at a certain value $p<0.05$ statistical significance was observed: p=0.0008, calc. F=11.7711, crit. F=3.6823. The Tukey HSD multiple comparison test revealed statistical differences between the categories of stroke/TIA and stroke/TIA with Nattokinase, at $p<0.01$ and between stroke/TIA with Nattokinase and those with oncological comorbidity, $p<0.01$, HSD [0.05] = 26.96; HSD [0.01] = 35.8 (Tables 2 and 3). Comparison among control patients and a certain value $p<0.05$ a statistical significance was also found: p=0.0009, calc. F=11.7327, crit. F=3.6823. The differences are related to the groups of recurrent cerebrovascular events and a total number of stroke/TIA with ASA/Clopidogrel at $p<0.01$, and stroke/TIA with ASA/Clopidogrel and group with concomitant malignant disease, $p<0.01$, HSD [0.05] = 27.25; HSD [0.01] = 35.86 (Tables 4 and 5).

Within a 6-month follow-up period, transient hematuria (1) and cases of gastrointestinal discomfort (2) were noted in patients receiving Nattokinase. In the control group with standard antithrombotic prevention, epistaxis (1), skin changes with the character of ecchymoses (1) and gastrointestinal complaints (2) were reported. The ratio of possible adverse reactions between the two categories is 3.13%:4.21%, statistically insignificant (p=0.64, calc. t=0.52, crit. t=3.18) and confirms the conclusion of the literature and own initial data for a safe profile of the enzyme Nattokinase [23-26].

Despite the serious limitations of the study (a small number of selected patients, short observation period, lack of information about ASA and Clopidogrel resistance in control group participants etc.) there is no statistically reliable difference with the occurrence of vascular (brain and cardiac) complications between the two groups. Patients who take Nataspin H® had a higher incidence of premorbid oncological conditions. One possible explanation is related to the associated mechanisms of hypercoagulation that have led to changes in blood viscosity and the development of arterial thrombosis – essential factors in the pathogenesis of ischemic disorders of the cerebral circulation [27].

Conclusion

The concept of Nattokinase as an alternative of standard prevention is interesting, original and promising, in line with the current trends for an interdisciplinary approach and the principles of personalised medicine.

Acknowledgments

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