

Research Article

EXPERIMENTAL EVALUATION OF THE EFFECT OF ENDOTHELIN RECEPTOR ANTAGONIST ON NATTOKINASE INDUCED CHANGE IN HEART RATE AND BIOCHEMICAL PARAMETERS IN THE RAT

Anand R, A Soosai Manickam* and Manoj G Tyagi

**Department of Pharmacology and Physiology, Christian Medical College, Vellore 632002, Tamilnadu*

**Author for Correspondence*

ABSTRACT

Nattokinase is a potent fibrinolytic enzyme, obtained from 'natto' a traditional food in Japan. It breaks fibrin strands both by hydrolyzing fibrin in blood clots directly and hastens the production of tissue plasminogen Activator. It was also found to be lower the blood pressure and has been used in the management of atherosclerosis, coronary artery disease, and stroke. Endothelin-1 (ET-1) is a very potent vasoconstrictor and binds to smooth muscle endothelin receptor. ET-1 has been shown to be released by the failing myocardium where it can contribute to cardiac calcium overload and hypertrophy. In this study we conducted studies to evaluate the cardiac rate and some biochemical parameters to ascertain the interaction between endothelin antagonist BQ-123 and nattokinase.

Key Words: *Nattokinase, Bq-123, Hypertension, Heart Rate, Blood Urea Nitrogen*

INTRODUCTION

Nattokinase was first found and extracted from 'natto', which is a boiled and fermented soyabean a traditional food in Japan (Maruyama and Sumi, 1995 and Sumi *et al.*, 1987). Nattokinase is a potent fibrinolytic enzyme that effectively breaks down fibrin strands and thrombi. Nattokinase can both hydrolyze fibrin in blood clots directly and hasten the production of t-PA (Tissue Plasminogen Activator), which activates plasminogen into active plasmin to hydrolyze fibrin (Sumi *et al.*, 1990). Decreasing blood viscosity strikes at the root of arteriosclerosis and atherosclerosis as well as hypertension, peripheral vascular disease and congestive heart failure¹. The fibrinolytic activity of nattokinase resolves the active process of atherosclerosis and lyses the thrombi. The per oral administration along with prolonged half-life of 4-6 hours and extremely safe profile show favourably upon nattokinase as the key agent for restoration of vasculature health (Sumi *et al.*, 1990 and Kim *et al.*, 2008). While on the other hand the vasoconstrictor peptide, Endothelin-1 (ET-1) is a 21 amino acid peptide that is produced by the vascular endothelium. It is a very potent vasoconstrictor that binds to smooth muscle endothelin receptors, of which there are two subtypes: ETA and ETB receptors (Schiffirin and Touyz, 1998). These receptors are coupled to a Gq-protein and receptor activation leads to the formation of IP3, which induces the release of calcium by the sarcoplasmic reticulum (SR) and increased smooth muscle contraction and vasoconstriction. An increase in endothelin may cause altered cardiovascular hemodynamics (Gulati *et al.*, 1996). There are also ETB receptors located on the endothelium that stimulate the formation of nitric oxide, which produces vasodilation in the absence of smooth muscle ETA and ETB receptor activation. This receptor distribution helps to explain the phenomenon that ET-1 administration causes transient vasodilation (initial endothelial ETB activation) and hypotension, followed by prolong vasoconstriction (smooth muscle ETA and ETB activation) and hypertension. ET-1 receptors in the heart are also linked to the Gq protein and IP3 signal transduction pathway. Therefore, ET-1 in the heart causes SR release of calcium, which increases contractility. ET-1 also increases the heart rate (Bascon *et al.*, 1996). Endothelin receptor antagonists, by blocking the vasoconstrictor and cardiotoxic effects of ET-1, produce vasodilation and cardiac inhibition. Endothelin receptor antagonists have been shown to decrease mortality and improve hemodynamic in experimental models of heart failure (Barker *et al.*, 2001). Congestive heart failure is a disease process characterized by impaired left ventricular function, increased peripheral and pulmonary vascular resistance and sodium and water retention. Since the prevalence of

Research Article

CHF increases, it will be a major cause of morbidity and mortality in the future. Heart failure with normal or minimally impaired systolic function is attributed to diastolic dysfunction and is termed diastolic heart failure (DHF). Hypertension induces, thickening of ventricular wall and leads to ventricular hypertrophy, which decreases ventricular pressure gradient and decrease left ventricular filling (Grossman *et al.*, 1975). The nattokinase enzyme has been found to be beneficial for patients of hypertension. Traditional knowledge is that ‘natto’ in the diet tends to lower blood pressure. It has also been suggested that the mechanism for this effect may be the inhibition of ACE. Endothelin antagonism may also be helpful in alleviating the condition of heart failure and improve cardiovascular hemodynamics (Dashwood *et al.*, 1994) with these findings; in this study we evaluated interaction of endothelin receptor antagonist BQ-123(5mg/kg) with nattokinase (25mg/kg) in the cardiovascular system and biochemical parameters.

MATERIALS AND METHODS

Animals

Experiments were performed on male Wistar Albino rats of weighing, between 200-250g obtained from experimental animal centre of Christian Medical College, Vellore, India. Animals were housed in groups of 3-4 in polypropylene plastic cages under hygienic conditions, lined with paddy-husk bedding. Animals were housed in a colony room once the experiments completed under controlled temperature (25+/-degree), relative humidity of (60+/-2%) and were exposed to 12 hour light: 12 hour dark cycle, with food and water available *ad libitum*. All experiments were conducted during the light phase, between 8.00-13.00 hours. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC).

Heart Rate Measurement

The animals were anesthetized using ketamine (10mg/kg i.m) and Diazepam (4mg/kg i.p). Electrocardiography was conducted using the limb lead II on a physiograph (INCO, India) using a speed of 10 mm/second for the control reading. Nattokinase (25mg/kg, i.p) was administered to the animal intraperitoneally and for every 15 minutes interval electrocardiography was conducted on a physiograph using a speed of 10 mm/second. To evaluate the effect of ET-1 antagonist, the experiment was conducted in another group of animals with BQ-123 (5mg/kg, i.p) treatment. And finally to evaluate the combination effect of Nattokinase with Endothelin receptor antagonist both Nattokinase (25 mg/kg, i.p) and BQ-123 (5mg/kg, i.p) were administered in combination and the electrophysiology was conducted. Heart rate was estimated from the ECG tracings by counting the number of ‘R’ waves per minute as per earlier described technique (Tyagi and Thomas, 1999).

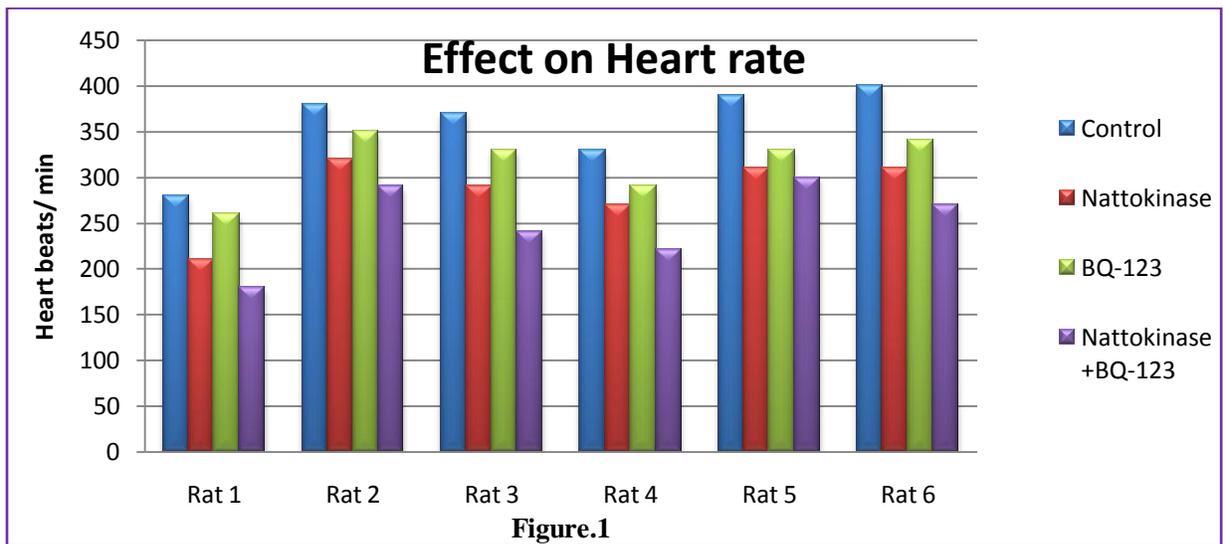


Figure 1: Effect of nattokinase and BQ-123 on heart rate in rats

Research Article

Estimation of Blood Urea Nitrogen (BUN)

To evaluate the Blood Urea Nitrogen level, the animals were treated with nattokinase and BQ-123. After 60 mins of drug treatment blood samples were collected by retro orbital sinus under light ether anaesthesia and the serum were separated by centrifuge with 2500 rpm for 10 mins at 4^o C. Then the blood urea nitrogen levels were measured using auto-analyzer. The values were shown in Table 1.

Table 1: Effect of nattokinase and BQ-123 on blood urea nitrogen levels

Drugs	BUN(mg/dl)	1	2	3	4	5
Control		33.09	33.16	30.29	32.16	31.23
Nattokinase		27.50	28.03	27.50	26.56	26.56
BQ-123		28.89	28.43	30.29	30.29	28.89
Nattokinase + BQ-123		15.84	17.71	20.97	17.60	19.57

Estimation of Blood Glucose

For the measurement of blood glucose the animals were treated in a same manner with nattokinase and BQ-123 and after 60 mins, the blood sample was collected from the tail vein without anaesthesia and monitored the glucose using Glucometer (Abbott,USA). The values were shown in Table 2.

Table 2: Blood glucose level

Drugs	Sugar(mg/dl)	Before Treatment	After Treatment
Control		116	118
Nattokinase		94	96
Endothelin antagonist		114	113
Nattokinase + Endothelin antagonist		100	98

RESULTS

The effects of nattokinase enzyme on heart rate are shown in Fig. 1, and in this study the heart rate was reduced when compared to control heart rate. The endothelin receptor antagonist BQ-123 when injected alone did not alter much the reduction in heart rate, but when given in combination with nattokinase there was significant reduction in heart rate when compared to nattokinase alone.

Similar to the heart rate the Blood Urea Nitrogen level which is shown in Table 1, there is a significant reduction of BUN with nattokinase and also alongwith BQ-123. Hence the combination of Nattokinase and BQ-123 shows much more reduction in BUN level as compared to control levels.

The blood glucose levels did not show any appreciable difference between the control and drug treated groups.

DISCUSSION

Hypertension is the most common risk factor for the heart failure. It leads to left ventricular (LV) hypertrophy, which decreases LV compliance and LV diastolic filling and is associated with diastolic heart failure. Thus there is a strong co-relation between persistent hypertension and cardiac failure.

Nattokinase in the diet has been found to lower the blood pressure and also it has been used in managing atherosclerosis, coronary artery disease, stroke, peripheral vascular disease (Sumi *et al.*, 1990). The possible mechanism for reducing blood pressure is possibly by inhibiting angiotensin – converting enzyme (ACE), because the antihypertensive peptides which reduce the ACE was also found in ‘natto’. Hence in this study we confirmed that nattokinase exhibited a significant reduction in heart rate (Okamoto *et al.*, 1995).

ET-1 is a very potent vasoconstrictor that binds to smooth muscle endothelin receptors, of which there are two subtypes: ETA and ETB receptors, which in turn increased to leads to pulmonary hypertension and

Research Article

congestive heart failure. ET-1 in the heart causes SR release of calcium, which increases contractility. Endothelin antagonist has been useful in hypertension patients.

We also evaluated the effect of BQ-123 in this study, which caused enhanced reduction in the heart rate in combination with nattokinase. This study shows the combination of the nattokinase with endothelin antagonist, BQ-123 may be a useful in treatment in reducing heart rate. The blood urea nitrogen was found to be a powerful marker for mortality in acutely decomposed heart failure. BUN was also increased due to low cardiac output and with renal impairment. The use of diuretics, ACE inhibitors or Angiotensin receptor blocks contributes a treatment in decreasing BUN (Aronson, 2004; Kirtane *et al.*, 2005 and Smith *et al.*, 2006).

In our study, we found an interesting co-relation of heart rate reduction with BUN level in both nattokinase alone and in combination along with endothelin antagonist. The multi-pronged effect on including anti-atherosclerotic, vasodilatory and negative chronotropic action can be exploited for these cardiovascular ailments (Tyagi, 2012). Hence our findings suggest that this combination of nattokinase and BQ-123 may be useful for the treatment of heart failure and other cardiovascular dysfunctions as suggested in a previous article (Pais *et al.*, 2006).

ACKNOWLEDGEMENT

The authors thank the clinical Biochemistry department, CMC Vellore for their help in the estimation of Blood urea nitrogen.

REFERENCES

- Aronson D, Mittleman MA and Burger AJ (2004).** Elevated blood urea nitrogen level as a predictor of mortality in patients admitted for decompensated heart failure. *American Journal of Medicine* **116** 466-473.
- Bacon CR, Cary NRB and Davenport AP (1996).** Endothelin peptide and receptors in human atherosclerotic coronary artery and aorta. *Circulation Research* **79** 794-801.
- Barker S, Khan NQ, Wood EG and Corder R (2001).** Effect of an antisense oligodeoxynucleotide to endothelin-converting enzyme-1c (ECE-1c) on ECE-1c mRNA, ECE-1 protein and endothelin-1 synthesis in bovine pulmonary artery smooth muscle. *Molecular Pharmacology* **59** 163-169.
- Dashwood MR, Allen SP, Luu TN and Muddle JR (1994).** The effect of the ETA receptor antagonist, FR139317, on [¹²⁵I]-ET-1 binding to the atherosclerotic human coronary artery. *British Journal of Pharmacology* **112** 386-289.
- Grossman W, Jones D and McLaurin LP (1975).** Wall stress and patterns of hypertrophy in the human left ventricle. *Journal of Clinical Investigation* **56**(1) 56-64.
- Gulati A, Sen AP, Kumar A and Tyagi MG (1996).** Increase in endothelin and removal of nitric oxide contribute towards the efficacy of diaspirin crosslinked hemoglobin in hemorrhaged rats. Abstract, Art. Cells Blood Subs. *Immobil. Biotechnology* **24**(4) 345.
- Kim JY, Gum SN, Paik JK, Lim HH, Kim KC, Ogasawara K, Inoue K, Park S, Jang Y and Lee JH (2008).** Effects of nattokinase on blood pressure: a randomized, controlled trial. *Hypertens Resource* (8) 1583-1588.
- Kirtane AJ, Leder DM, Waikar SS, Chertow GM, Ray KK, Pinto DS, Karmaliotis D, Burger AJ, Murphy SA, Cannon CP, Braunwald E and Gibson CM (2005).** Serum blood urea nitrogen as an independent marker of subsequent mortality among patients with acute coronary syndromes and normal to mildly reduced glomerular filtration rates. *Journal of the American College of Cardiology* **45** 1781-1786.
- Marsh WH, Fingerhut B and Miller H (1965).** Automated and manual direct methods for the determination of blood urea. *Journal of Clinical Investigation* **11** 624.
- Maruyama M and Sumi H (1995).** Effect of Natto Diet on Blood Pressure. *JTTAS*.

Research Article

Okamoto A, Hanagata H, Kawamura Y and Yanagida F (1995). Anti-hypertensive substances in ferment- ed soybean, natto. *Plant Foods for Human Nutrition* **47** 39-47.

Pais E, Alexy T, Holsworth RE Jr and Meiselman HJ (2006). Effects of nattokinase, a pro-fibrinolytic enzyme, on red blood cell aggregation and whole blood viscosity. *Clinical Hemorheology and Microcirculation* **35**(1-2) 139-142.

Schiffrin EL and Touyz RM (1998). Vascular biology of endothelin. *Journal of Cardiovascular Pharmacology* **32** Supply 3 S2-S13.

Smith GL, Shlipak MG, Havranek EP, Foody JM, Masoudi FA, Rathore SS and Krumholz HM (2006). Serum urea nitrogen, creatinine, and estimators of renal function: mortality in older patients with cardiovascular disease. *Archives of Internal Medicine* **166** 1134-1142.

Sumi H, Hamada H, Nakanishi K and Hiratani H (1990). Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. *Acta Haematol* **84**(3) 139-143.

Sumi H, Hamada H, Tsushima H, Mihara H and Mureki H (1987). A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese natto: A typical and popular soybean food in the Japanese diet. *Experienta* **43**(10) 1110-1111.

Tyagi MG and Thomas M (1999). Enhanced cardiovascular reactivity to desmopressin in water-restricted rats: facilitatory role of immunosuppression. *Methods Find Exp. Journal of Clinical Investigation* **21**(9) 619-624.

Tyagi MG (2012). A combination therapy of nattokinase enzyme and endothelin receptor antagonists for treating hypertension and cardiac failure? *JPBMS* **21** (09).