Thrombosis Research 131 (2013) 158–161

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Antithrombotic effect of taurine in healthy Japanese people may be related to an increased endogenous thrombolytic activity

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ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form 17 September 2012
Accepted 25 September 2012
Available online 24 October 2012

Keywords:
Taurine
Fish
Sea Food
Thrombosis
Fibrinolysis
Cardiovascular diseases

ABSTRACT

Introduction: Prevention of arterial thrombotic diseases has high priority in developed countries. Taurine (2-aminomethylsulfonic acid), which is rich in sea foods, showed antithrombotic effect in animal models of thrombosis. The present study aimed to investigate such effect in healthy human volunteers.
Methods and Results: In 101 healthy Japanese people the overall thrombotic status was accessed from non-anticoagulated blood sample by the Global Thrombosis Test (GTT). There was no significant correlation between taurine concentration in urine samples and GTT-Occlusion Times (OT; mainly reactivity of platelets). In contrast, a significant inverse correlation was demonstrated between urine taurine concentrations and GTT-Lysis Times (LT; showing spontaneous thrombolytic activity).
Conclusions: Our findings suggest that taurine enhances endogenous thrombolytic activity which could be a mechanism of the earlier observed cardioprotective and antithrombotic effect.

Introduction

The preventive effect of fish and sea-food eating on myocardial infarction and stroke has long been recognized. Intake of marine animals containing eicosapentaenoic and docosahexanoic acids, the precursors of prostaglandins, prevented arterial thrombogenesis both in animal models and in humans [1]. Further, several studies demonstrated that a free amino acid taurine (2-aminomethylsulfonic acid), which is rich in sea foods had protective effect against heart attacks and strokes [2–7]. Although such effect of taurine is largely attributed to its free radical scavenging antioxidant effect and alteration of calcium and magnesium metabolism, the exact mechanism of the antithrombotic effect of taurine is still unknown. The most intriguing observation was the profound cardioprotective effect of taurine. Administered before or after ischemia or during reperfusion, taurine prevented re-infarction and reduced myocardial injury [8–10]. Although there are reports showing inhibition of agonist-induced platelet aggregation by taurine [11,12], this cannot be the full explanation for the cardioprotective effect.

In this study we used a new point-of-care test the Global Thrombosis Test (GTT). This test allows for the first time, the assessment of all components of thrombotic/haemostatic status including platelet reactivity, (platelet-dependent) coagulation and endogenous thrombolytic activity from one native blood sample. As impeded endogenous thrombolytic activity has recently been shown as a hidden and important player in acute coronary syndromes [13], we attributed great importance to the assessment of endogenous thrombolytic activity in people on taurine-rich diet.

The thrombotic status of a cohort of volunteers on taurine-rich diet was assessed by this global technique GTT and the recorded thrombotic parameters were compared with indices measured from serum and urine samples.

Materials and Methods

Methods

Healthy Volunteers

101 healthy volunteers (aged 32–76 years, 27 male and 74 female) not taking regular medications including antiplatelet, antirheumatic

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http://dx.doi.org/10.1016/j.thromres.2012.09.021

drugs or anticoagulants were enrolled after informed consent. This study was approved by the institutional ethics committee of Mukogawa Women's University and by the institutional ethics committee of Ishii Hospital.

Physical and Physiological Examination

Blood pressure and heart rate were measured by sphygmomanometer (HEM-907, Omron, Kyoto, Japan) in the rested sitting position. Body fat mass and body fat ratio were measured by bioelectrical impedance analysis (HBF-302, Omron, Kyoto, Japan) in the rested standing position.

Blood Sampling

Blood was collected after 12-h fast from antecubital vein using 21 G butterfly needle (Top Corporation, Tokyo, Japan) from 9:00 am to 12:00 am in an air conditioned room (25 ± 0.5 °C). The first 11 ml blood was used for routine blood tests, while the subsequent 2 × 3.0 ml non-anticoagulated (native) blood was used to perform the Global Thrombosis Test.

Global Thrombosis Test (GTT)

The principle of GTT and the technique have been described in details elsewhere [14,15]. The GTT test tube has a conical part, into which two balls are placed. Because of four flat segments formed on the inner surface of the conical part of the tube, there are four narrow gaps by the balls. When blood is added to such tube it flows by gravity through the narrow gaps by the balls and collected in a reservoir. Flowing through the gaps by the upper and larger ball, platelets are exposed to high shear stress and activated. In the space between the two balls, platelet aggregates are formed, thrombin is generated from activated platelets and such fibrin-stabilized thrombi gradually occlude the narrow gaps by the lower ball thus arresting the blood flow. The instrument detects the time interval (d, sec) between consecutive blood drops falling into the reservoir. At the start of the test, blood flow is rapid and hence (d) is small. Subsequently, the flow rate gradually decreases and hence (d) increases. When the actual $d \geq 15$ sec (occlusion-d), the instrument records this time (T1 sec.) and displays as 'Occlusion Time; OT'. Later, flow is completely arrested. There is also an arbitrarily preset time (200 sec) following OT, during which the sensors are inactive. This time is to allow stabilization of the formed thrombi and ignore eventual small re-bleeds. After this time, due to endogenous thrombolysis, eventually flow is restored as indicated by detection of the first blood drop after OT + 200 sec (T2) and this is displayed as Lysis Time (LT sec). Hence, Lysis Time (LT) = T2-T1. GTT is unique in respect of assessing thrombolysis as opposed to fibrinolysis, thus taking into account of the contribution of platelets to the increased resistance of arterial thrombus to lysis [16].

Measurement of Thrombotic Biomarkers

Blood samples were anticoagulated by heparin-coated tube. Plasma was separated by centrifugation at 1500 × g for 20 min at 4 °C. The markers were analyzed by Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

Urine Collection and Tests

24-h urine was collected using an 'aliquot cup', which collected 1/40 of the total urine volume. Urinary taurine, isoflavone and K^+/Na^+ were measured by an amino acid analyzer (Hitachi 835, Ibaragi, Japan), high performance liquid chromatography (Japan) and atomic spectrophotometry (Japan), respectively.

Statistical Analysis

Correlations were analyzed by the Pearson test. Values were expressed as means ± SD. P value less than 0.05 was considered as limit of significance. The commercially available statistical software Unistat (version 4.53, London, UK) was used.

Results and Discussion

Tables 1 and 2 show the volunteers' baseline characteristics and the relationship with GTT Lysis Times (LT) and Occlusion Times (OT). From the listed components only HDL cholesterol and spot urea creatinine were significantly correlated with OT, urinary taurine and LT values (Table 2). Importantly, a significant (inverse) correlation was found between taurine concentration in urine (as a marker of seafood intake [17]) and LT but not with OT (Fig. 1).

Urinary taurine levels were in a wide range, the explanation for this may be the fact that the people in the Cohort came from various locations [4].

Taurine affects platelets, the major players in arterial thrombosis. Platelets contain high concentration of taurine and its reduction caused platelet hyperaggregability [18]. Further, taurine inhibited ADP-induced platelet aggregation and reduced the mortality rate in mice from thrombosis induced by intravenous injection of ADP or collagen-epinephrine mixture [11,12]. Contrary to the expectation, we could not demonstrate any significant relationship between urinary taurine concentrations and platelet reactivity (OT) as measured by GTT.

In contrast to platelet function, very little data are available for the effect of taurine on fibrinolysis. One early publication claimed taurine as a fibrinolytic agent in humans [19]. Taurine and its derivatives accelerated t-PA-induced plasma (fibrin) clot lysis and t-PA-catalyzed plasminogen activation [20]. Impaired endogenous thrombolytic activity in acute coronary syndrome patients predicted cardiovascular death and nonfatal myocardial infarction [14]. Thus our present findings of a significant (inverse) correlation between

Table 1
Baseline characteristics of study population.

Basal data	(mean ± SD)
Age (year)	51.3 ± 7.7
Height (cm)	160.4 ± 8.3
Weight (kg)	57.2 ± 10.0
BMI (kg/m2)	22.1 ± 3.0
Body fat (%)	26.5 ± 5.9
Body fat mass (kg)	15.2 ± 4.8
Waist circumference (cm)	76.0 ± 8.8
SBP (mmHg)	120.5 ± 19.8
DBP (mmHg)	71.6 ± 12.9
Heart rate	66.7 ± 10.1
Occlusion time (OT, sec)	537.8 ± 103.1
Lysis time (LT, sec)	2106.3 ± 974.5
Blood sample data	**(mean ± SD)**
Blood glucose (mg/dl)	90.9 ± 13.3
HbA1C (%)	5.0 ± 0.6
Insulin (μIU/ml)	4.2 ± 2.4
Triglyceride (mg/dl)	96.4 ± 60.7
Total cholesterol (mg/dl)	209.3 ± 31.6
HDL cholesterol (mg/dl)	64.8 ± 15.1
Urine sample data	
Urinary isoflavone (μmol/day)	35.6 ± 40.4
Urinary potassium (mEQ/l)	35.4 ± 13.9
Urinary sodium (mEQ/l)	120.3 ± 45.5
Urinary creatinine (mg/dl)	85.7 ± 42.0
Spot urea potassium (mEQ/l)	26.5 ± 18.3
Spot urea sodium (mEQ/l)	115.0 ± 49.9
Spot urea creatinine (mg/dl)	90.0 ± 53.4
Urinary volume (ml/day)	1353.4 ± 621.3
Creatinine coefficients (mg/kg/day)	17.5 ± 6.1
Sodium chloride volume (g)	8.8 ± 4.0
Sodium potassium ratio (%)	3.7 ± 1.6
Urinary taurine (μmol/day)	940.0 ± 976.1