Nerve Growth Factor Receptors in Cardiovascular Disease

Manoochehr Messripour

Abstract— Nerve growth factor (NGF) modulates synaptic transmission between sympathetic neurons and promotes cardiac repair following myocardial infarction (MI). Reports indicated that NGF receptor expression is down regulated to a trace detectable level during the postmaturation phase, but pathological factors stimulate the NGF receptors (NGFR) synthesis, and release the extra cellular parts of the receptors as truncated soluble forms (TNGFR) to biological fluids. Although the role of the sympathetic nervous system in regulating heart rate is very well established, little is known about the effect of NGF on the myocardial infarction.In this study we determined TNGFR in urine samples collected from 18 hospitalized patients with heart attack. The clinical presentation and serum troponin 1 concentration confirmed severe MI. Healthy volunteers (n=18) with no clinical evidence of cardiac disease were included as control. Concentrations of TNGFR in the urine samples were determined by a quenching fluoroimmunoassay.The mean levels of TNGFR in the urine samples of healthy control subjects and the patients were 114± 23 and 371± 66 ng/mg creatinine respectively ng/mg creatinine respectively. The TNGFR level the urine samples from one patient was not different from that of control subjects. The levels of TNGFR in the patient samples decreased to near control values 3 weeks post-heart attack.The results confirmed the previous observations that pathological factors may express relatively high levels of NGF receptors of the cardiac myocytes.

***Keywords*—** Cardiac myocytes, Myocardial infarction, Nerve growth factor, Troponin.

I. Introduction

NERVE growth factor (NGF) modulates synaptic transmission between sympathetic neurons and cardiac myocytes. It has been suggested that NGF promotes cardiac repair following myocardial infarction (MI) ,[1] - [2]. The specific biological functions of NGF actions are modulated via the specific NGF receptors of the target cells [3] - [4] Several lines of evidence indicated that NGF receptor expression is down regulated to a trace detectable level during the postmaturation phase, but pathological factors stimulate the NGF receptors (NGFR) synthesis, and release the extra cellular parts of the receptors as a truncated soluble form to biological fluids [5], [6], [7].

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An increased level of NGFR has been reported in patients with neurodegenerative diseases [5], [6], [7]. It has been demonstrated that cardiac-specific overexpression of NGF leads to sympathetic hyperinnervation of heart [8], [9], [10]. Several lines of evidence indicated that target-organ responses to sympathetic nervous stimulation are related to the synaptic concentration of two neurotransmitters; norepinephrine (NE) and NGF. In vitro studies demonstrated that NE regulates synthesis and secretion of NGF in cultured mouse astroglial cells , while; NGF modulates synaptic transmission between sympathetic neurons and cardiac myocytes [11]. Although the adverse pathophysiological effects of cardiac sympathetic over activity following myocardial infarction (MI) are increasingly recognized, little is known about the process by which sympathetic neurons from synaptic activity between these neurons and NGFR of the cardiac myocytes . Because cardiac troponin 1 is used as a marker of myocyte injury the present study was undertaken to determine the relation serum cardiac troponin 1 and alterations of urinary excretion of TNGFR in patients with cardiovascular diseases.

II. Materials And Methods

Chemicals Nerve-growth factor (purified 75MW, NGF), Fluorescein insufficient isomer 1 (FITC), Acryl amide, bis-acrylamide, DEAE-cellulose, CM-cellulose, Tween 80, Freund's adjuvant (complete and incomplete), Sephadex G-25, G-100, G-200 fine grade were purchased from Sigma (Poole, Dorset, UK). ELISA diagnostic kit for determination of troponin 1.Unless stated otherwise, all reagents were of the highest grade and made up in double glass-distilled water.

Patients and Samples Hospitalized patients (n=18) following heart attack were asked to participate in this study voluntarily. The clinical presentation and diagnostic investigations confirmed severe MI by cardiac specialists. All patients underwent cardiologic evaluation and receiving conventional therapies. Healthy volunteers (n=18) with no clinical evidence of cardiac disease were included as a control population. Age (48-76 year) and sex were matched. The first morning blood and urine samples were collected and serum cardiac troponin I and urine NGFR were assayed in the same day. The assays were repeated 3weeks post-heart attack.

Determination of troponin 1 ELISA were incubated in a 96-well plate precoated by monoclonal anti-troponin 1

antibody. After washing, alkaline phosphatase conjugated antibody was added. The wells were washed again and followed by the addition of substrate. Light production which was proportional to the concentrations of troponin was measured in relative luminescence units, using a microplate reader with chemiluminescence capability.diagnostic kit was used to measure troponin I in the collected serum samples. Briefly, roponin 1 standard and samples

Fluorescein-Labeled NGF Preparation of F-NGF was prepared as described by Messripour and Moin [12]. Equal volumes of FITC solution (1 mg/ml) and purified NGF solution (about 0.25 mg protein/ml) were mixed and stirred overnight at 4 °CA The entire labeled NGF was separated from unconjugated FITC using Sephadex G-25 column (1.2×20 cm).

Fluoroimmunoassay of NGFR The concentrations of NGFR were measured by fluoroimmunoassy essentially as described by Messripour and Moein [12]. Briefly, fluorescein-Labeled NGF (100 □l) was added to triplicate tubes containing 100 □l of urine samples. After 5 min 100 □l of a 1:10 diluted anti-fluorescein serum (rabbit) in saline was added, and after another 15 min incubation at room temperature, the volume was increased up to 2 ml by addition of Tris buffer, and fluorescence intensities of the mixture were measured using a Parkin-Elmer (Norwalk, CT) LSE spectrophotofluorimeter with the excitation wavelength of 495 nm and an emission wavelength of 540 nm. In all experiments, a correction was made for the background signal contributed by the reactions other than that of FNGF. The amount of NGFR is expressed as ng/ml of FNGF which remained fluorescent. All samples were run in duplicate, and the average value is reported.

Gel diffusion method Agarose gel diffusion of NGF against urine samples was carried out as described by Ouchterlong [13]. Agarose (1%) in buffer containing NGF was layered on a plastic plate. The urine samples taken from diagnosed patients and apparently normal subjects were pipetted into the gel wells. The plates were placed in the cold room for 72 and the radial band around the wells was considered as positive.

Statistical analysis The obtained data were subjected to statistical analysis using SPSS software (version 18). In all cases, the one-way analysis of variance (ANOVA) was used to compare the mean of each group with the control group. The LSD complementary test was conducted to elucidate the exact differences at p-value lower than 0.05. Data are presented as mean ± standard deviation.

III. Results

The concentrations of urinary TNGFR and serum troponin I in patients with MI and the control groups are shown in table I. The mean levels of TNGFR in the urine samples of the patients with MI and healthy control subjects were 371± 66 ng/mg creatinine 114± 23 and respectively. The data for the patients was more than 3 folds greater than that recorded from the healthy control subjects. The differences are statistically significant ($p< 0.05$). The mean serum concentration of troponin 1 in corresponding samples was 11.8± 5.5 and 0.066± 0.031 ng/l. The norma l range for troponin 1 is estimated to be around 0.1 ng/l [14]. Table II shows comparative studies of urinary TNGFR as assayed by both fluoroimmunoassay and agarose gel diffusion in different sub group of both the patients and healthy subjects. As can be seen in Table I, there is a relationship between the levels of TNGFR in different ages of both control and patient subgroups. The TNGFR level of the urine samples from one patient was not different from that of control subjects. The levels of TNGFR in the patient samples decreased to near control values 3 weeks post-heart attack.

TABLE I
COMPARISON OF TNGFR LEVELS IN URINE OF PATIENTS WITH MI AND HEALTHY SUBJECTS

Samples	TNGFR (ng /mg creatinine)	Troponin I (ng//ML)
Patient (n=18)	371± 66	11.8± 5.5
Healthy (n=18)	114± 23	0.066± 0.031

The levels of TNGFR and troponin I were assayed by FRA and ELISA in the urine and serum samples from patients with MI and healthy individuals respectively. The age and sex were matched. The results are mean ± separate determinations.

IV. Discussion

Enormous studies indicated that under certain clinical circumstances, most notably in cardiovascular disease, activation of the sympathetic nervous system plays a key role in the pathophysiology of the disease process [8], [9], [10]. Despite data indicating augmented release of NE from the failing heart, histological examination in cardiovascular diseases indicates that the innervations density of sympathetic neurons is reduced [8]. Therefore, paradoxical finding of high catechilamine release and the reduced sympathetic innervations density in the failing heart remains unexplained. In addition, higher level of NGFR observed in aging of both patients and healthy subjects have been implicated as underlying the general age-related changes to the myocards [14] Although the mechanism(s) leading to these alterations in the cardiac myocytes are not well understood, there are several reasons to suggest that two major harmful age-associated changes to the heart have been identified; first, fibrotic scarring on the endo- and epi-cardial surfaces and second,, age-related myocardial tissue remodeling, the mechanisms leading to age-related myocardial decline. Nevertheless, it has thus far been difficult to separate the effects of aging from those of age associated diseases such as; atherosclerosis and hypertension on cardiac performance. It appears that NGF in the sympathetic nervous system acts as a target-derived survival factor, supply in the limiting quantities by target organs to regulate the final number of neurons and density of innervations. Higher levels of NGFR observed in this study however, may be interpreted as being consistent with the up-regulation of NGFR in the

cardiac myocytes of in the patients with cardiovascular disease. It appears that NGFR expression in the heart is dynamic and may be stimulated by the disease states. This suggestion is in agreement with the over-expression of NGFR in neurodegenerative disorders [5], [6] , [7].

TABLE II
COMPARISON OF TNGFR LEVELS IN URINE OF PATIENTS WITH MI AND HEALTHY SUBJECTS

Samples	Age	TNGFR	Gel diffusion
PATIENTS (DAY 1)			
Group 1	46-56	276 ± 47 [4]	UN [1], ++ [3]
Group 2	57-66	353 ± 71 [6]	+ [2], ++ [4]
Group 3	67-76	388 ± 64 [8]	+++ [8]
PATIENTS (DAY 21)			
Group 1	46-56	207 ± 47 [4]	UN [4]
Group 2	57-66	227 ± 71 [6]	UN [4], WP [2]
Group 3	67-76	249 ± 64 [8]	+ [5], WP [3]
CONTROL			
Group 1	46-56	106 ± 18 [4]	UD [4]
Group 2	57-66	73 ± 12 [6]	UD [4], WP[2]
Group 3	67-76	202 ± 37 [8]	UD [6], WP[2]

The levels of TNGFR (ng /mg creatinine **)** were assayed either by fuoroimmunoassay and agarose gel diffusion in the urine samples from patients with patients with MI and healthy individuals. The age was matched and shown in different subgroup numbers in each subgroup are given in brackets. Data are presented as mean ± standard deviation. UD= Undetectable.

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