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Rapid Communication

Angiotensin Converting Enzyme Inhibitors Attenuated the Expression of G-Protein Coupled Receptor Kinases in Heart Failure Patients

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Short title: ACEI attenuates GRK expression

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Abstract

Lymphocyte GRK mRNA expression was measured by quantitative RT-PCR in 15 congestive heart failure patients: 5 in NYHA class-II treated with ACEI (IIA), 5 in NYHA class-II without ACEI (IIC) and 5 in NYHA class-III treated with ACEI (IIIA). GRK2 and GRK5 mRNA of IIIA were significantly higher than those in IIA. GRK mRNA expression level in IIA were significantly lower than those of IIC. These results suggest that the expression level of lymphocyte GRK may reflect the severity of CHF, and that ACEI treatment could reduce the level of GRK in CHF patients.

Key words: G protein-coupled receptor kinase, ACE inhibitor, congestive heart failure

Introduction

The function of G-protein coupled receptors is regulated via their phosphorylation by a group of serine-threonine protein kinases, G-protein coupled receptor kinases (GRK). So far, six different GRK family members have been cloned^(1,2). Among them, GRK2 and GRK5 are abundantly expressed in mammalian hearts⁽³⁾. We previously reported that the expression of these GRKs was markedly enhanced in several animal models of congestive heart failure (CHF), and their possible involvement in the desensitization process of β -adrenergic receptors (β AR) in failing hearts⁽⁴⁻⁶⁾. We also reported that the expression level of GRK mRNA in peripheral lymphocytes was closely correlated with that in hearts⁽⁷⁾. The purpose of this study is 1) to investigate the correlation between the expression level of GRK mRNA in peripheral lymphocytes and NYHA class, and 2) to investigate the effect of angiotensin converting enzyme inhibitors (ACEI) on GRK expression.

Methods

Among the CHF patients who admitted to the Hokkaido University Hospital between April 1999 and March 2002, fifteen patients were randomly selected for this study. Routine medical treatment was performed to stabilize their heart failure. After obtaining written informed consent, blood sample was obtained at various time points during the course of treatment. The lymphocyte fraction was separated from whole blood through Ficoll-Paque gradient centrifugation^(8,9), and total RNA was extracted from the lymphocyte fraction using the Single-Step method⁽¹⁰⁾ and then GRK mRNA was assessed by quantitative RT-PCR as previously described⁽⁷⁾. The intensities of DNA bands were assessed by densitometric scanning of photographs and used to calculate the relative expression level of GRK mRNA to that of GAPDH mRNA (AU, arbitrary unit) using image analyzing software, NIH image. Data were expressed as means \pm SD. Values were compared using unpaired *t*-test, and accepted as

statistically significant when p value was less than 0.05.

Results

Table 1 shows the baseline characteristics of the 15 CHF patients. In summary, the mean age of these patients was 63.4 years plus or minus 10.3 years. The New York Heart Association classifications (NYHA) were class II in 10 patients, and class III in 5 patients. ACEI were administered to 10 of the patients, and not to 5. β -blockers were administered to 6 patients and not to 9 patients.

As shown in Figure 1, NYHA class III CHF patients had substantially higher expression level of GRK2 and GRK5 mRNA in their peripheral lymphocytes than NYHA class II patients (GRK2; IIIA 1.11 ± 0.06 AU vs. IIA 0.73 ± 0.18 AU, $p < 0.05$, GRK5; IIIA 0.70 ± 0.20 AU vs. IIA 0.35 ± 0.04 AU, $p < 0.05$). ACEI substantially reduced the level of GRK2 and GRK5 in NYHA Class II patients (GRK2; IIA 0.73 ± 0.18 AU vs. IIC 1.54 ± 0.28 AU, $p < 0.05$, GRK5; IIA 0.35 ± 0.04 AU vs. IIC 0.69 ± 0.06 AU, $p < 0.05$). β -blockers did not affect the level of GRK mRNA (data not shown)

Discussion

These results clearly demonstrated that the expression level of GRK mRNA reflected the severity of CHF, and that ACEI abolished the enhanced expression of GRK. ACEI-mediated transcriptional regulation of GRK documents a novel mechanism of β -adrenergic receptor regulation, and may provide, at least in part, a potential mechanism responsible for the beneficial effect of ACEI in the treatment of CHF. To our best knowledge, this is the first report showing that GRK mRNA could be used as a marker for assessing the severity of heart failure. Conventional markers, such as ANP, BNP and catecholamines rise quickly when CHF deteriorates, and rapidly decrease in parallel with the healing process of

CHF. On the contrary, however, expression of GRK mRNA persists well into the recovery phase of CHF where hemodynamic stability has already been achieved⁽⁷⁾. This characteristic temporal profile of GRK expression is suitable to evaluate the severity of CHF even after successful treatment of hemodynamic instability of CHF patients. Obviously, it could be of great help for clinical diagnosis of CHF.

There are 2 limitations in this study. First, the small number of patients might fail to show the effect of β -blocker on the expression level of GRK2/5. Second, we have not tested the effect of angiotensin II receptor antagonists (ARB) on GRK expression, mainly because the period for patients enrollment of this study was ranging from 1999 to 2002. It is highly possible that ARB has the similar pharmacological effect to ACEI. Further study with larger number of patients in various clinical settings would be necessary to establish the clinical usefulness of GRK mRNA measurement.

We concluded that the expression of GRK mRNA in peripheral lymphocytes could be a new molecular marker, which reflects the severity of congestive heart failure. ACEI significantly reduce the expression of GRK mRNA, which might restore the reduced β -adrenergic receptor function in CHF patients.

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Figure Legends

Figure 1

Effect of the severity of CHF and ACEI on the levels of GRK2 and GRK5 mRNA

AU: arbitrary unit

Table 1 Characteristics of the CHF patients

NO.	Age	Sex	Diag.	NYHA	ACEI	β
1	53	F	VHD	II	-	-
2	74	F	VHD	II	-	-
3	66	M	cAf	II	-	+
4	53	F	VHD	II	-	-
5	67	F	DCM	II	-	+
6	52	M	DCM	II	+	-
7	66	M	ICM	II	+	+
8	70	F	ICM	II	+	-
9	57	M	DCM	II	+	-
10	67	F	DCM	II	+	+
11	75	F	RCM	III	+	-
12	72	F	VHD	III	+	-
13	38	F	DCM	III	+	+
14	71	M	ICM	III	+	-
15	60	M	DCM	III	+	+

Diag.: diagnosis, NYHA: New York Heart Association classification, ACEI: administration of angiotensin converting enzyme inhibitors, β : administration of β -blockers, RCM: restrictive cardiomyopathy, DCM: dilated cardiomyopathy, VHD: valvular heart disease, ICM: ischemic cardiomyopathy, cAf: chronic atrial fibrillation.

Figure 1

