Rapid Communication

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

Naotsugu Oyama, M.D., Kazushi Urasawa, M.D., Satoshi Kaneta, M.D.,
Hidetsugu Sakai, M.D., Takahiko Saito, M.D., Chika Takagi, M.D., Ichiro Yoshida, M.D.,
Akira Kitabatake, M.D. and Hiroyuki Tsutsui, M.D.

Department of Cardiovascular Medicine
Hokkaido University Graduate School of Medicine, Sapporo, Japan

Short title: ACEI attenuates GRK expression

This research is supported in part by a Research Grant from the Ministry of Health and Welfare of Japan, Grants-in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (06454283, 06557041).

Address for correspondence;
Kazushi Urasawa, M.D., Ph.D.
Department of Cardiovascular Medicine
Hokkaido University Graduate School of Medicine
Kita 15, Nishi 7, Kita-ku
Sapporo Hokkaido 060-8638, Japan

TEL 81-11-706-6973
FAX 81-11-706-7874
E-mail: kazurasa@med.hokudai.ac.jp

Total number of pages: 10
Total number of figures: 1
Total number of tables: 1

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 (βAR) 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 Ficall-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 densitmetric 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 ± 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.

References

1. Chuang TT, Iacovelli L, Sallese M, De Blasi A. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. ***Trends Pharmacol Sci*** 1996; 17: 416-421.
2. Haga T, Haga K, Kameyama K. G protein--coupled receptor kinases. ***J Neurochem*** 1994; 63: 400-412.
3. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. Beta-Arrestin: a protein that regulates beta-adrenergic receptor function. ***Science*** 1990; 248: 1547-1550.
4. Urasawa K, Sato K, Igarashi Y, Kawaguchi H, Yasuda H. A mechanism of catecholamine tolerance in congestive heart failure - alterations in the hormone sensitive adenylyl cyclase system of the heart. ***Jpn Circ J*** 1992; 56: 456-461.
5. Urasawa K, Yoshida I, Takagi C, Onozuka H, Mikami T, Kawaguchi H. et al. Enhanced expression of beta-adrenergic receptor kinase 1 in the hearts of cardiomyopathic Syrian hamsters, BIO53.58. ***Biochem Biophys Res Commun*** 1996; 219: 26-30.
6. Takagi C, Urasawa K, Yoshida I, Kaneta S, Nakano N, Onozuka H. et al. Enhanced GRK5 expression in the hearts of cardiomyopathic hamsters, J2N-k. ***Biochem Biophys Res Commun*** 1999; 262: 206-210.
7. Oyama N, Urasawa K, Kaneta S, Sakai H, Saito T, Takagi C, Yoshida I, Kitabatake A, Tsutsui H. Chronic β–adrenergic Receptor Stimulation Enhanced the Expression of G-Protein Coupled Receptor Kinases, GRK2 and GRK5, both in Hearts and Peripheral Lymphocytes. ***Circ J*** 2005; 69: 987-90.
8. Lombardi MS, Kavelaars A, Schedlowski M, Bijlsma JW, Okihara KL, Van de Pol M. et al. Decreased expression and activity of G-protein-coupled receptor kinases in peripheral blood mononuclear cells of patients with rheumatoid arthritis. ***FASEB J*** 1999; 13:

715-725.

9. Boyum A. Isolation of leukocytes from human blood. A two-phase system for removal of red cells with methylcellulose as erythrocyte-aggregating agent. ***Scand J Clin Lab Invest*** 1968; 97 suppl: 9-29.
10. Chomczynski P, Sacci N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. ***Anal Biochem*** 1987; 162: 156-159.

Figure Legends

Figure 1

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

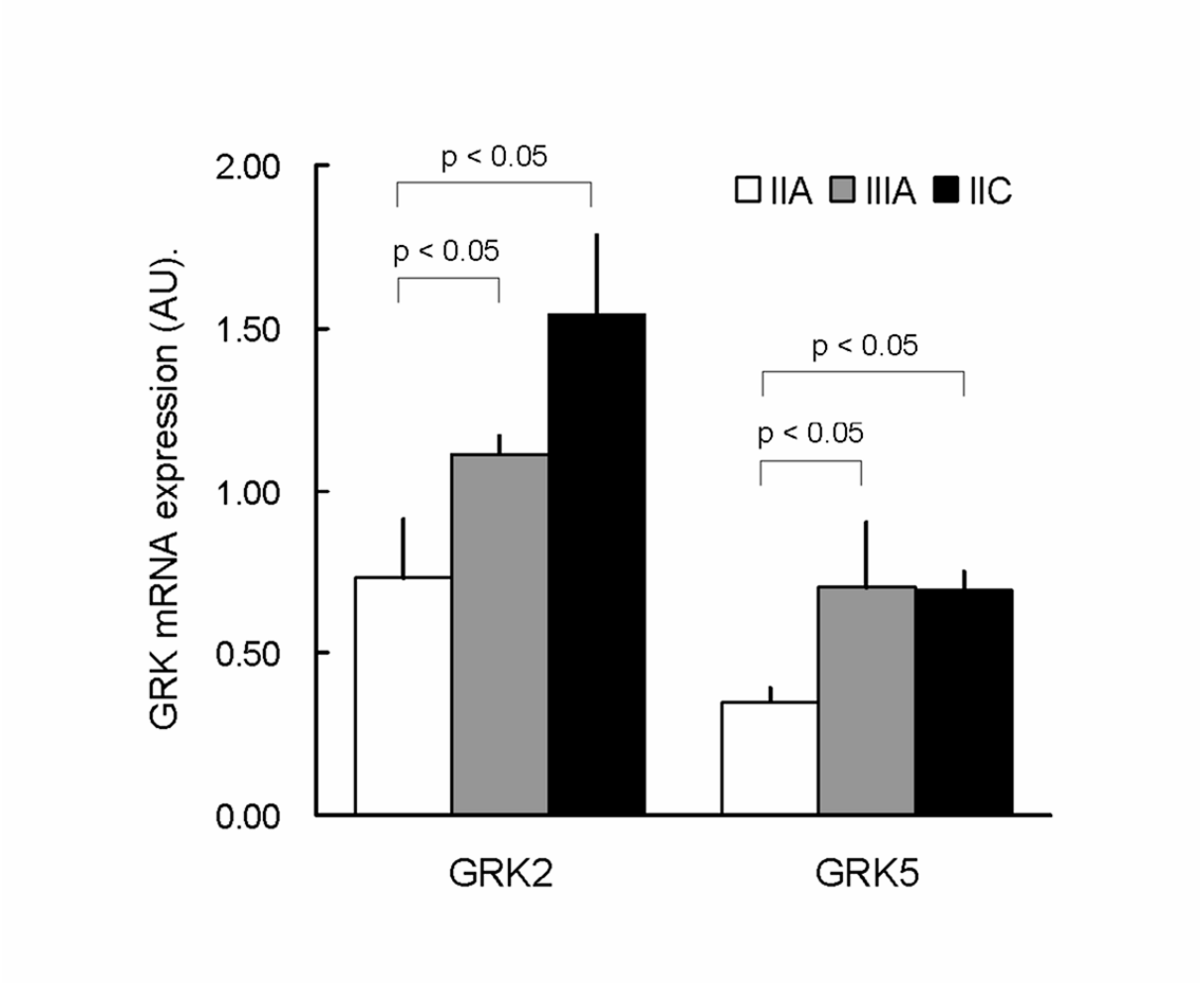
AU: arbitrary unit

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


p < 0.05
p < 0.05
IIA IIIA IIC
2.00
1.50
1.00
0.50
0.00
GRK mRNA expression (AU).
p < 0.05
p < 0.05
GRK2
GRK5