The Increase in β -Adrenergic Receptors in Lymphocytes Following Treatment with Xamoterol

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Xamoterol (100 mg b.d.), a β_1 -adrenergic partial agonist, was given orally for 7 days to 8 healthy volunteers and to 8 patients with heart failure. β -adrenoceptors in lymphocytes were determined by radioligand-binding assay using ^{125}I -iodocyanopindolol before and after the administration of xamoterol.

The values of density ($B_{\max}$) and affinity (K_d) of the β -adrenoceptors were lower in the patients with heart failure than in the healthy volunteers ($B_{\max}=931\pm214$ vs 1466 ± 373 sites/cell, and $K_d=0.60\pm0.11$ vs 1.07 ± 0.14 nM). During treatment with xamoterol, the values of $B_{\max}$ ($7,169\pm3,768$ and $7,749\pm3,807$ sites/cell) and K_d (6.01 ± 3.84 and 9.06 ± 4.66 nM) increased strikingly ($P<0.01$) in both groups. Following withdrawal of the treatment, the values of $B_{\max}$ and K_d decreased in both groups, but $B_{\max}$ of the patients with heart failure remained higher ($1,385\pm457$ sites/cell, $P<0.05$) than the pre-treatment level. Blood pressure rose slightly in the patients with heart failure during treatment with xamoterol, but heart rate and fractional shortening on echocardiography were unaffected.

In conclusion, β -adrenoceptors in lymphocytes were up-regulated during treatment with xamoterol without deterioration of cardiac function in the patients with heart failure because of the β -agonist and β -antagonist activities of the drug.

Key words: xamoterol, β -adrenoceptor, heart failure, β_1 -partial agonist

Introduction

Quantitative and qualitative changes in receptors have recently been studied since advances in radioligand binding assay and purification of receptor proteins.^{1,2)} There is evidence that changes in β -adrenoceptors in the myocardial membrane and lymphocytes in heart failure are closely related to the pathophysiology and treatment of the con-

dition.³⁾

Norepinephrine is released from the sympathetic nerve endings. It affects myocardial β -adrenoceptors and increases heart rate and ventricular contraction. However, in patients with chronic heart failure, the myocardium may be damaged by elevated plasma norepinephrine (NE), and myocardial β -adrenoceptors may be down-regulated progressively. Thus, β -blockade may be attempted to up-regulate the β -adrenoceptors in patients with heart failure, but this therapy is still controversial.^{4,5)} Therefore, it is hoped that a drug which up-regulates β -adrenoceptors and improves cardiac function will be

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developed.

In the present study, xamoterol (ICI 118, 587), a partial agonist for β_1 -adrenoceptor, was given to healthy volunteers and to patients with heart failure, and the characteristics of β -adrenoceptors in the lymphocytes were assayed before and after the treatment.

Subjects

1. Control group

Xamoterol (100 mg b.d.) was given orally for 7 days to 8 healthy male volunteers aged 18 to 23 yr with a mean age of 20 yr. Blood pressure, heart rate, β -adrenoceptors in lymphocytes, and fractional shortening (FS) on M-mode echocardiography were evaluated before the treatment, on days 2, 5, and 7 of the treatment, and again 2 and 5 days after withdrawal of the treatment.

2. Heart failure group

Xamoterol was given orally at 100 mg b.d. for 7 days to 8 patients with heart failure at degree II or III of the New York Heart Association (NYHA) criteria, who had been stable for more than one month. They were 46 to 65 years old with a mean age of 58 yr. Three were females. Dilated cardiomyopathy was found in 6 and valvular heart disease in 2. They were tested before and after the treatment as were the healthy volunteers. The cardiothoracic ratio (CTR) was $68 \pm 8\%$. Digitalis, diuretics, and vasodilators were also given during the study without any change in the regimens. No patient received β -agonists or β -antagonists concurrently.

The study was carefully explained to all healthy volunteers and the patients with heart failure, and all gave their informed consent.

Methods

1. Blood NE concentrations

Pre-treatment blood NE concentrations at rest were determined in the healthy volunteers and in the patients with heart failure. The determination was made early in the morning, by high-performance liquid chromatography with electrochemical detection, using our modification.⁶⁾

2. Blood concentrations of xamoterol

Blood concentrations of xamoterol were determined by radioimmunoassay at 2 hr after treatment

with xamoterol.

3. Fractional shortening (FS)

M-mode echocardiograms were recorded using a phased array electronic sector scanner at a frequency of 2.4 MHz (Model SSH-11 A. Toshiba Co.), and FS was calculated by the following formula:

$$FS = (\text{diastolic-systolic}) / \text{diastolic left ventricular dimension.}$$

4. Isolation of lymphocytes and binding assay

In all subjects of both groups, β -adrenoceptors in lymphocytes were determined by radioligand-binding assay. Heparinized 15 ml blood samples were drawn, laid on Ficoll conray according to Böyum's method,⁷⁾ and centrifuged at 1,500 rpm for 30 min to isolate the lymphocytes. These were washed twice with phosphate-buffered saline at 1,000 rpm for 10 min, and suspended in a mixture of 120 mM Tris-HCl and 40 mM $MgCl_2$ at pH 7.4. The lymphocytes were incubated at 23°C for 60 min together with ^{125}I -iodocyanopindolol (ICYP) in 60 mM Tris-HCl and 20 mM $MgCl_2$ at pH 7.4. Incubation was stopped by filtration through a glass fiber filter (GF/C, Whatman). Radioligand bound to lymphocytes was determined with an Autowell gamma counter (ARC-251, Aloka). The lymphocytes were incubated with and without 10 μM l-propranolol, and the radioligand binding suppressed by propranolol was defined as the specific binding to β -adrenoceptors. The radioligand was used at 6 to 12 concentrations (0.01–1.0 nM), and the results were analyzed by Scatchard plot, and then density (B_{max}) and affinity (K_d) of the β -adrenoceptors were calculated.⁸⁾ Proteins were assayed by Lowry's method,⁹⁾ and the number of β -adrenoceptors per lymphocyte cells was calculated by the method of Galant et al.¹⁰⁾

Statistical Analysis

All data are expressed as the mean $\pm$ 1 standard deviation and differences in means were assessed by Student's t-test. Values of $P < 0.05$ were considered significant.

Results

1. Blood NE concentrations (Tab.)

Blood NE levels were higher ($P < 0.05$) in the

Tab. Effects of Xamoterol on Hemodynamics and β -Receptors in the Control and the Heart Failure Groups

	Pre-treatment	Xamoterol			Withdrawal	
		2	5	7 (days)	2	5 (days)
(A) Healthy volunteers						
Norepinephrine (ng/ml)	0.23±0.08					
Drug concentration (ng/ml)	—	84±23	61±35	69±24	<5	<5
Heart rate (beats/min)	78±15	81± 7	75±13	86±13	78±11	73±11
Systolic BP (mmHg)	118±17	127±17*	133±19*	126±16*	122±16	120±15
Fractional shortening (%)	35±3	38±4*	39±3*	39±3*	36±4	35±3
B _{max} (sites/cell)	1,466±373	7,169±	4,946±	5,130±	1,998±214*	1,420±383
		3,768**	2,500**	2,889**		
K _d (nM)	1.07±0.14	6.01±	3.55±	2.61±	0.99±	1.01±
		3.84**	2.30**	1.15**	0.21	0.16
(B) Heart failure						
Norepinephrine (ng/ml)	0.51±0.29					
Drug concentration (ng/ml)	—	73±20	88±31	82±37	<5	<5
Heart rate (beats/min)	70±9	72±11	75±7	71±8	74±8	76±8
Systolic BP (mmHg)	110±13	115±11	127±16*	121±11*	117±5	116±11
Fractional shortening (%)	19±7	19±7	20±8	20±9	20±9	19±8
B _{max} (sites/cell)	931±214	7,749±	4,293±	4,125±	1,696±	1,385±
		3,807**	2,160**	1,928**	435**	457*
K _d (nM)	0.60±0.11	9.06±	2.73±	2.56±	1.63±	0.61±
		4.66**	2.16**	1.67**	0.70**	0.14

BP, blood pressure; B_{max} and K_d, density and affinity of the β -adrenoceptors, respectively. Values are mean $\pm$ 1 SD.

* P<0.05 and ** P<0.01 when compared with values before xamoterol.

Fig. 1 Results of a typical binding isotherm. Left panel shows a saturation-binding curve. Intact lymphocytes were incubated with various concentrations of the β -adrenergic antagonist 125 I-iodocyanopindolol (ICYP), and the binding was determined with filtration to separate bound and unbound radioactivity. The experiment was performed both in the presence of 10 μ M propranolol to determine nonspecific binding (nonspe) and in its absence to determine total binding (total). The difference between the two values represents specific binding (specific) to the receptors. Right panel shows a Scatchard plot. The specific binding results from left panel are transformed in order to plot the ratio of bound to free radioactivity against the amount bound. In this linear plot, the intercept yields the maximum number of binding sites (B_{max}) and the slope is the negative reciprocal of the dissociation constant (K_d).

patients with heart failure (0.51 ± 0.29 ng/ml) than in the healthy volunteers (0.23 ± 0.08 ng/ml). Levels higher than 0.60 ng/ml— 0.68 ng/ml, and the other with 0.74 ng/ml— were detected in 2 patients with NYHA III.

2. Blood concentrations of xamoterol (Tab.)

Blood concentrations of xamoterol were high (84 ± 23 and 73 ± 20 ng/ml) in day 2 of the treatment in both the healthy volunteers and the patients with heart failure. The drug was eliminated from the blood after withdrawal of the treatment.

3. Blood pressure, heart rate, and FS before and after treatment with xamoterol (Tab.)

Blood pressure rose slightly during the treatment in both the healthy volunteers and the patients with heart failure, but heart rate was unaffected. FS increased slightly during the treatment in the healthy volunteers, but it returned to the pre-treatment level after withdrawal of the treatment. In the patients with heart failure, FS was much lower than in the healthy volunteers (19 ± 7 vs $35 \pm 3\%$, $P < 0.05$) and was not affected by the drug.

4. Basic evaluation by binding assay (Fig. 1)

ICYP bound to lymphocytes reached the peak level after about 15 min, and then a constant level was maintained for up to 60 min. When $10 \mu\text{M}$ propranolol was added, it was dissociated from the lymphocytes, indicating that the binding was reversible. When the dose of ICYP increased in incubation, ICYP bound to the lymphocytes increased accordingly. However, the binding was saturated at a certain level, and Scatchard plot showed a straight line.

5. β -adrenoceptors in lymphocyte following treatment with xamoterol (Tab. and Fig. 2)

As shown in the table, lower values of B_{max} (931 ± 214 vs $1,466 \pm 373$ sites/cell, $P < 0.05$) and K_d (0.60 ± 0.11 vs 1.07 ± 0.14 nM, $P < 0.05$) of the lymphocytes of the heart failure group than those of the healthy group were observed. The values of B_{max} ($7,749 \pm 3,807$ and $7,169 \pm 3,768$

Fig. 2 Effects of xamoterol administration on the density (B_{max} =sites/cell) and affinity (K_d =nM) of the lymphocyte β -adrenoceptors in the control and the heart failure groups. Each point is the mean of values from all subjects. * $P < 0.05$ and ** $P < 0.01$ vs pre-treatment days.

sites/cell) and K_d (9.06 ± 4.66 and 6.01 ± 3.84 nM) of the two groups increased strikingly on day 2 of the treatment as compared to the pre-treatment level (both $P < 0.01$). On days 5 and 7, these values remained higher than those of the pre-treatment level. Although these values of the healthy group returned to the pre-treatment level after withdrawal of the treatment, the value of B_{max} of the heart failure group was still higher than that of the pre-treatment level ($1,385 \pm 457$ sites/cell, $P < 0.05$).

Discussion

β -adrenergic full agonists are effective acutely in improving heart failure, but lose their efficacy in long-term therapy because of decrease in density of myocardial β -adrenergic receptors, i.e. down regulation.¹¹⁾ Therefore, cardiotonics such as dibutyryl cyclic AMP which do not increase cyclic AMP through β -adrenoceptor are clinically investigated but their long-term efficacy has been

disappointing.¹²⁾

Interestingly, β -adrenergic antagonists are sometimes effective in improving heart failure. The efficacy of the antagonists may be explained by improved diastolic performance of the myocardium, protection of the myocardium from increased catecholamine levels, and restoration of β -adrenoceptor sensitivity for catecholamines. In general, the antagonists are, however, likely to aggravate heart failure and are used in the treatment of heart failure in only a few centers.^{13,14)} Thus, attention has recently been directed to the use of adrenoceptor partial agonists.¹⁵⁾

Xamoterol is a partial agonist for β_1 -adrenoceptor; its intrinsic sympathomimetic activity is 43% that of the level of isoprenaline.¹⁶⁾ When the sympathetic tone is low, it acts as a β_1 -agonist ($\beta_1 : \beta_2 = 1,000 : 1$), and when the sympathetic tone is high, it acts as a β -antagonist; the cardioselectivity ratio ($\beta_1 : \beta_2$) as an antagonist is 13 : 1 in the dog and 40 : 1 in the rat and rabbit.^{16,17)} Thus, xamoterol will stabilize the cardiac function against changes in sympathetic tone. Xamoterol may be useful in patients with heart failure who show abnormally elevated sympathetic tone and whose β -adrenoceptors are down-regulated.¹⁸⁾

For convenience, β -adrenoceptors in lymphocytes have been commonly used for studies of down-regulation in heart failure.^{19,20)} Although whether lymphocyte β_2 -adrenoceptor changes correspond to the changes in myocardial β_1 -adrenoceptor is still controversial.^{21,22)} The cardiovascular effects of xamoterol are mediated by β -adrenoceptors located on myocardium and/or vascular smooth muscle. Thus, the striking increase in lymphocyte β -adrenoceptor density during xamoterol treatment raises the possibility that the observed hemodynamic attenuation was due to an increase in the density of β -adrenoceptors on myocardium and/or vascular smooth muscle. There is no information on the density of β -adrenoceptors in the hearts. However, lymphocytes from patients with heart failure and marked elevated levels of plasma norepinephrine failed to generate normal amounts of cyclic AMP in response to isoproterenol.²²⁾ Consequently, it was suggested that "down regulation" of β -adrenoceptors might occur in such patients because of chronic sympathetic stimulation, which in turn appears related to the severity of left ventricular dysfunction.^{22,23)} The signifi-

cantly lower β -adrenoceptor density in our patients with heart failure before xamoterol treatment, as compared with that in healthy volunteers, is consistent with this hypothesis.

In the present study, xamoterol increased the values of $B_{\max}$ and K_d of lymphocyte β -adrenoceptors in healthy volunteers and patients with heart failure. These results suggested that xamoterol up-regulated β -adrenoceptors in both healthy volunteers and patients with heart failure, and this effect seemed to be related to the β -antagonist effect of the drug. In the healthy volunteers, the drug increased blood pressure and FS, and these results seemed to be related to the β -agonist effect of the drug. In the patients with heart failure, heart rate and FS were unaffected, but blood pressure rose, suggesting that the drug exerted a mild β -agonist effect even in patients with heart failure when plasma norepinephrine levels were about 0.51 ng/ml.²⁴⁾

In our previous study, the acute and chronic effects of xamoterol were evaluated in patients with heart failure in NYHA class II or III.^{18,24)} When xamoterol was given intravenously, blood pressure, heart rate, and cardiac index were all unaffected acutely. Nevertheless, exercise tolerance, hemodynamics and arrhythmias improved when the drug was given for a long-term period. These results indicated that xamoterol was ineffective in heart failure when given acutely, but gradually improved the condition when given over a prolonged period.

The present study showed that the values of $B_{\max}$ of lymphocyte β -receptors increased in patients with heart failure in NYHA class II or III following treatment with xamoterol, without adversely affecting cardiac function. This suggests that xamoterol may improve cardiac function by an appropriate effect of antagonist activity (preventing the cardiotoxic effect of catecholamines, normalizing down-regulated β -adrenergic pathway, suppressing exercise-induced tachycardia) and an agonist activity (increasing ventricular contractility).

Treatment of xamoterol caused an increase in the values of K_d in the human lymphocyte β -adrenoceptors from both healthy volunteers and patients with heart failure in the present study. β -adrenoceptor agonists influenced the values of K_d in insulin receptor sites and prostaglandins caused

the changes in the signaling system of β -adrenoceptors.²⁵⁻²⁷ The signaling system using cyclic AMP as the second messengers may be the best known, and β -adrenoceptors conformed to this system.²⁸ It is possible that xamoterol caused the changes of the coupling systems between β -adrenoceptors, GTP-binding protein, and adenylate cyclase.

We suppose that these combined effects of xamoterol, when given chronically to patients with heart failure, result in up-regulation of myocardial β -adrenoceptors, in improved cardiac function, decrease in plasma norepinephrine levels, and decrease in arrhythmias.

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