Changes in Myocardial $\beta_1$-Adrenergic Receptor and Stimulatory G-Protein Gene Expression after Chronic Treatment with Doxorubicin in Rat

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ABSTRACT. The gene expression of $\beta_1$-adrenergic receptor ($\beta_1$AR) and stimulatory G-protein Gs\textgreek{a} in ventricle after chronic treatment with doxorubicin (DOX) in rat was investigated. The rats were treated with DOX in a dose of 2.5 mg/kg once a week for 5 weeks, the cumulative dose being 12.5 mg/kg. Two weeks after the last injection, the positive inotropic effect of isoproterenol was noticeably decreased in left atrial muscle preparations isolated from DOX-treated rats. Northern blot hybridization showed that the mRNA transcripts of $\beta_1$AR and Gs\textgreek{a}, important signal transduction elements for regulating heart rate and contractility, were significantly decreased in the ventricle of DOX-treated rats. Thus, chronic treatment with DOX decreases the gene expression levels of myocardial $\beta_1$AR and Gs\textgreek{a}.

KEY WORDS: $\beta_1$-adrenergic receptor, doxorubicin, G-protein.

Anthracyclines such as doxorubicin (DOX) are potent chemotherapeutic agents with wide clinical applications in the treatment of neoplasms, but life-threatening cardiotoxicity often develops and is a major limiting factor for further use of DOX [16]. The mechanisms of DOX cardiotoxicity include the production of reactive oxygen species and membrane lipid peroxidation, change in cardiac energy production, direct DNA damage and interference with DNA repair, and change in Ca$^{2+}$ handling by the sarcoplasmic reticulum [6, 7, 12, 14, 17].

The $\beta_1$-adrenergic receptor ($\beta_1$AR) is a principal subtype of $\beta$-adrenergic receptors ($\beta$AR) regulating heart rate and contractility [1]. Stimulation of cardiac $\beta_1$AR by catecholamines results in the activation of the stimulatory G-protein Gs\textgreek{a}, which in turn, activates the adenyl cyclase and promotes the production of cAMP. Chronic treatment with DOX reduced $\beta$-adrenergic receptor density and basal or agonist-stimulated adenyl cyclase activity in the myocardium [2, 15, 19]. Nevertheless, the precise mechanisms of $\beta$AR and post-receptor abnormalities remain unclear. Boucek et al. [3] suggested that a progressive DOX effect on cardiac gene expression was a possible mechanism to consider. Therefore, we investigated the gene expression of $\beta_1$AR and Gs\textgreek{a} in rat ventricular muscle after chronic treatment with DOX.

Male Wistar rats (7 to 9 weeks-old, Nihon Clea, Tokyo, Japan) were housed in standard cages, and were maintained on a standard laboratory diet and tap water and exposed to a 12-hr light-dark cycle. Ambient temperature during the study was maintained at about 23°C. After all animals used in the present study had been cared for in accordance with the guidelines for animal treatment of Kitasato University, which meet the principals of laboratory animal care, DOX (Kyowa Hakko, Tokyo, Japan) was administered intravenously once a week, 2.5 mg/kg a week for 5 weeks. The control rats were injected with physiological saline (1 ml/kg) in the same regimen. Two weeks after the last injection, the rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). The hearts were immediately excised and perfused via the aorta with a Langendorff apparatus with Krebs-Henseleit bicarbonate buffer solution (118 mM NaCl, 27.2 mM NaHCO$_3$, 4.8 mM KCl, 1.0 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 1.2 mM CaCl$_2$ and 11.1 mM glucose). The solution was saturated with a 95% O$_2$ and 5% CO$_2$ gas mixture, yielding a pH value of 7.4 at 30°C. After all visible blood was washed out, the hearts were separated into the atrial and ventricular tissues. The left atrial tissues were used in in vitro experiments on cardiac muscle contraction. The ventricular tissues were frozen immediately in liquid nitrogen and stored at −80°C for RNA extraction. The most predominant feature of rats in the DOX group was the development of dyspnea. At the time of sacrifice, pleural effusion in the thoracic cavity was noted in the DOX-treated rats. Although none of the control rats died during the study, a mortality of 40% was observed in the DOX group in the 2 weeks post treatment. Body weight was significantly lower in the DOX group than in the control group (Table 1). Furthermore, DOX treatment resulted in a significant decrease in ventricle weight and ventricle weight/tail length ratio. This reduction in body weight gain due to DOX is attributed to a reduction of food intake and inhibition of protein synthesis [18]. The tail length of a rat is a better indicator of growth than body weight in a condition of malnutrition. Therefore, we used the ventricle weight/tail length ratio as an evaluative parameter for DOX-induced cardiotoxicity. The significant decrease in the ventricle weight/tail length
ratio observed in the present study revealed cardiotoxic effects of chronic DOX consistent with other reports [12, 20].

To evaluate the effect of chronic DOX treatment on cardiac function, we examined the positive inotropic effect of isoproterenol (ISO) in left atrial muscle preparations isolated from control and DOX-treated rats (Fig. 1). The preparations were electrically stimulated at 2 Hz with square-wave pulses of 5 msec duration at a voltage approximately 50% above the threshold with a pair of platinum field-stimulation electrodes [5]. The resting tension was adjusted to 1.0 g. Developed tension was continuously monitored with a force-displacement transducer (model TB651T, Nihon Koden Kogyo, Tokyo, Japan) and a Power Lab 4/20 computer (ADInstruments, Colorado Springs, Co, U.S.A.). The concentration-response curves for ISO were generated on the preparation of control and DOX groups. Basal developed tension before the addition of ISO was significantly different in the control (369.4 ± 141.6 mg) and DOX groups (92.9 ± 45.9 mg). In the atrial preparation of the control group, the positive inotropic effect of ISO was increased in a concentration-dependent manner. On the other hand, developed tension was markedly reduced in the DOX group at all the concentrations of ISO. These results, including body and ventricle weight, contractile profile, and gross observation indicate that chronic treatment of rats with DOX caused heart failure.

To examine the myocardial β1AR and Gsα gene expression after chronic DOX treatment, we used Northern blot hybridization (Fig. 2). To prepare the digoxigenin (DIG)-labeled cRNA probes, the polymerase chain reaction (PCR) primers for rat Gsα (GenBank accession number, M12673, 5’-AAGTGATCCAGTGTCTCAATGAT-3’ and 5’-TCCACGGCGAGGTAAAGTG-3’) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank accession number, M17701, 5’-GCTGATGCCCCCATGTTTG-3’ and 5’-GCCATAGGTCCACCC-3’) were designed. The β1AR primer sequences (5’-TCGTGTGACCGTGTGGGCC-3’ and 5’-AGGAAACGCGCTCAGCTGTCG-3’) were obtained from Krief et al. [10]. Then PCR was performed with ventricular cDNA as a template, and amplified cDNA products were subcloned into pGEM-T Easy vector (Promega, Madison, MI, U.S.A.). The resultant plasmids were linearized with Nco I or Sal I followed by transcription with T7 or SP6 RNA polymerase to generate the antisense or sense probe as described previously [9]. Five µg of total RNA extracted from ventricles of the control and DOX groups were fractionated on a 1.2% (w/v) agarose/formaldehyde gel. RNA was then transferred onto a positively charged nylon filter, and hybridized with a DIG-labeled cRNA probe as previously described [9]. DIG-labelled antisense β1AR, Gsα and GAPDH cRNA probes detected each mRNA transcript in the ventricles of the control group (Fig. 2A). No signal was obtained when a sense probe was used during hybridization (data not shown). The intensity was determined by the densitometric analysis of the autoradiogram. After normalization of each mRNA level to GAPDH mRNA levels, the bands corresponding to β1AR and Gsα mRNA were significantly decreased in the DOX group compared with the control (Fig. 2B and C). These results indicate that β1AR and Gsα gene expression in ventricles were decreased by chronic treatment with DOX.

DOX exhibits acute and chronic cardiotoxicity and has
been used to induce heart failure in various animal species. Acute cardiotoxic effects, such as hypotension, tachycardia and various arrhythmias, develop within minutes after administration of the drug [16]. These early effects are usually transient and clinically manageable, but do not constitute a major concern, but life-threatening chronic effects, including cardiac dilation and ventricular failure, develop several weeks or months after repeated DOX administration. In the present study, we selected chronic DOX treatment at the cumulative dose of 12.5 mg/kg (once a week with 2.5 mg/kg for 5 weeks), because it has been reported that this dosage regimen causes a significant cardiotoxicity in rats, and is considered to be a good model of DOX-induced chronic cardiotoxicity in humans [12, 13]. We found in the present study that chronic treatment with DOX induced cardiototoxicity similar to that in earlier reports [2, 12, 13, 15, 19, 20]. It has been reported that chronic DOX treatment caused reduction of $\beta_1$AR density on myocardial membrane, which is measured by using radioligand binding assay [2, 15, 19]. Although chronic treatment with DOX causes changes in the $\beta_1$AR system in the myocardium has been known for several years, changes in the $\beta_1$AR system-related gene expression have not been described. In the present study, we have demonstrated that the gene expression of $\beta_1$AR and Gs$\alpha$ in rat ventricle were decreased with chronic treatment with DOX. It is possible that reduction in $\beta_1$AR and Gs$\alpha$ mRNA may result in loss of expression of these proteins. Furthermore, it has been reported that basal and agonist-stimulated adenylyl cyclase activities were also decreased in myocardium treated with DOX [4, 15]. In general, the level of cAMP production is closely linked to $\beta_1$AR and Gs$\alpha$ protein content. Therefore the reduced myocardial $\beta_1$AR and Gs$\alpha$ mRNA after chronic treatment with DOX may cause a reduction in cAMP production, and then elicit dysfunction of cardiac contraction.

It has been reported previously that DOX decreases the expression of specific genes, including atrial and brain natriuretic peptide, troponin I, myosin light chain 2 and $\alpha$-actin, in promoting its cardiotoxic effects [3, 8]. The precise molecular mechanism underlying this inhibition of gene transcription is not clear, but has been suggested to be linked to a reduction in the MyoD transcription factor [11]. But it is not known whether the 5'-flanking promoter region of $\beta_1$AR or Gs$\alpha$ gene contains a MyoD responsive element, and the present study does not directly address the question of the mechanism of DOX effects on the reduction of $\beta_1$AR and Gs$\alpha$ gene expression. Further studies on promoter analysis are necessary to understand the gene regulation of $\beta_1$AR and Gs$\alpha$ in relation to DOX-induced cardiotoxicity.

The present results have indicated that abnormalities of the $\beta$-adrenergic receptor system in the myocardium of DOX-treated animals may stem, at least in part, from a decrease in the gene expression of $\beta_1$AR and Gs$\alpha$. 

Fig. 2. Expression of $\beta_1$AR and Gs$\alpha$ mRNA in ventricles of DOX-treated rats. Representative Northern blot of $\beta_1$AR, Gs$\alpha$ and GAPDH in the ventricles of the control and DOX groups (A). Densitometric analysis of $\beta_1$AR (B) and Gs$\alpha$ (C) mRNA expression in the ventricles. The levels were normalized to GAPDH. Data are given as the mean ± S.E.M. (n=4–5). *p<0.05 as compared to the control group.
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REFERENCES