CHAPTER V

DISCUSSION

In the present study, concentrations of DOX required to produce significant effects on Wistar rat atrial muscle preparations *in vitro* were substantially higher than its concentration in plasma found in human patients suffering from toxic effects of DOX. This is probably because atrial muscle preparations were exposed to DOX for a relatively short time, whereas human hearts are exposed chronically to this drug. The results of acute effect of DOX indicated that rate and force of atrial muscle preparations obtained from rat heart were depressed when the drug was added to the incubation medium *in vitro*. This result was similar to Viglione et al. (1992), who found that DOX caused a progressively depression of atrial rate in guinea pig. DOX produced negative inotropic effect was confirmed by several investigators (Hofling and Bolte, 1981; Politi et al., 1985; Singal and Pierce, 1986; De Jong et al., 1990; Voest et al., 1994; Hagane et al., 1988). Negative inotropic effects of DOX had been proposed by suppression of mitochondrial respiratory function and loss of ATP and creatine phosphate from the myocardium. Hagane et al. (1988) reported the depressed contractility of guinea pig atrial muscle preparation induced by DOX with affecting utilization of Ca^{2+} from the SR.

In atrial muscle preparation, Ca^{2+} for excitation-contraction coupling and contractile activation was derived from two distinct sources: Ca^{2+} influx across the sarcolemma and intracellular Ca^{2+} associated with SR (Sleator et al., 1964; Langer, 1968; Hajdu, 1969). Although Ca^{2+} from both sources was involved in contractile activation, Ca^{2+} from the SR was predominant in potentiated post-rest contractions (Nayler and Merrillees, 1971). The present study showed that acute treatment with DOX caused a decrease in PRC of left atria during the 3-hour experiment period, which was similar to the study of Hagane et al. (1988). Therefore, this result indicated that DOX

affected the amount of Ca^{2+} in the SR. Some studies was consistent with this finding that DOX altered the Ca^{2+} release function of the SR by effects on the Ca^{2+}-ATPase and the Ca^{2+} release channel. There was an evidence of binding of photoaffinity labelled DOX to the Ca^{2+} release channel in fractions enriched terminal cisternae, which indicated that DOX triggered Ca^{2+} release from skeletal SR vesicles (Zorzato et al., 1986). Others reported the effects of DOX on SR Ca^{2+} such as DOX increased the open probability of Ca^{2+} release channel reconstituted in lipid bilayers (Holmberg and Williams, 1990), induced Ca^{2+} release from cardiac SR vesicles (Kim et al., 1989; Pessah et al., 1990). Arai et al. (2000) reported that DOX inhibited the transcription of SERCA2 gene and induced down-regulation of the SR Ca^{2+}-ATPase 2 (SERCA2) gene expression by activating 3 MAPKs, which was a key transcriptional inhibitor of the SERCA2 gene.

Although the study of Politi et al. (1985) and Rasmussen et al. (1989) showed the interaction of DOX as a competitive beta-adrenergic receptor, the present results of acute effect of DOX on cumulative dose-response curve of isoproterenol showed that DOX depressed the maximal responses (Pmax) of isoproterenol in right and left atrial muscle preparations. This result was similar to that of Viglione et al. (1992). The decreased maximal responses of isoproterenol in this study by DOX suggest that DOX might be a nonspecific inhibitor or modify a common pathway of adrenergic system. DOX had been reported to alter beta-adrenergic systems. These included a decrease in the responsiveness of heart muscle to beta-adrenoceptor antagonists (Jensen, 1986), a decrease in adenylate cyclase activity (Calderone et al., 1991), a decrease in tissue cyclic AMP concentrations (Azuma et al., 1981; Shenasa et al., 1990) and downregulation of myocardial beta-adrenergic receptors (Robison and Giri, 1986; Fujita et al., 1991; Tong et al., 1991).

To determine further action of DOX on beta-adrenergic receptor, the cumulative dose-response curve of isoproterenol of the tissue preincubated with propranolol was performed to compare with that of DOX. The result indicated that DOX caused a decrease in positive responses to isoproterenol, but did not block these responses as

propranolol. These evidences suggested that DOX did not act as a competitive antagonist of adrenoceptor and might have other mechanisms for inhibiting adrenergic pathway.

In this study, DOX caused a right-ward shift of the cumulative-concentration response curve for acetylcholine observed in isolated left and right atrial muscle preparations without the reduction of maximal negative inotropic and chronotropic effects. This result indicated that DOX might act as a competitive antagonist at muscarinic receptors in the rat atria. The similar result was observed in the study of Temma et al. (1992 and 1993) in guinea pig heart. They also found in the inotropic study that the apparent affinity of the muscarinic receptor for acetylcholine was reduced in the presence of DOX. Hara et al. (2000) also determined that DOX produced a direct anticholinergic effect on muscarinic receptors with antagonistic action on carbachol-induced negative inotropic effect in guinea pig atria. These evidences still indicated that DOX was a competitive antagonist at muscarinic receptors in the guinea pig heart. However, Chugun et al. (2001) found that DOX caused a right-ward shift of the dose-response curves for the negative inotropic effect of carbachol and the maximal negative inotropic effect observed with high concentrations of carbachol was significantly attenuated by DOX. This showed that DOX might have multiple actions on the muscarinic system. The negative inotropic effects of muscarinic agonists are reported to be mediated by activation of ligand-gated K^+ channels that are regulated by a GTP binding protein (Gi or Gk) (Pfaffinger et al., 1985; Pappano and Mubagwa, 1992). Therefore, it was possible that the reduction of the maximal negative inotropic effects of carbachol by DOX resulted from the disruption of this pathway.

In subacute effect of DOX of this present study, the subacute dose of DOX used in this study (5 mg/kg total) was lower than those reported to produce cardiotoxicity in rabbits or rats. This dose did not cause acute or subacute death during the treatment but caused a significant change in atrial muscle functions. DOX caused a decrease of percentage change in PRC of left atrial muscle preparation comparing with

control. This similar result was found in the study of Hagane et al. (1988). They also found that PRC reached an apparent plateau with a much longer quiescent period in preparations obtained from DOX-treated animals. This present result indicated that subacute DOX treatment of rats might depress the release of Ca^{2+} from SR.

The possible relationship between the effect of the DOX on the cardiac beta-adrenoceptor and cholinergic receptor function in vitro and the development of delayed cardiotoxicity in vivo has been investigated in the rat. No differences in response to both isoproterenol and acetylcholine were found on the right or left atrial muscle preparations of rats injected with subacute dose of DOX. The result of Rasmussen et al. (1989) indicated that the delayed cardiotoxicity induced by DOX was not mediated by an interference with the cardiac beta-adrenoceptor function, although DOX caused marked ECG changes 5 weeks after the medication. No decrease in beta-adrenergic receptor population was found (Shenasa et al., 1990; Calderone et al., 1991). Additionally, Fu et al. (1991) reported that the densities, the affinity and number of binding sites on the adrenoceptors and muscarinic receptors in cardiac cell membrane preparations were not altered in rats chronically treated with DOX for 9 weeks.

Cardiac ATPases were necessary in the hydrolysis of ATP into ADP (adenosine diphosphate) and inorganic phosphate (Pi). The energy receiving from the hydrolysis was used for the contraction and relaxation. If ATP or ATPase is lacking, insufficient energy may available for both contraction and relaxation (Hamlin, 1999). In this study, treated rats with 5 mg/kg total DOX had no changes in either myosin ATPase or actomyosin ATPase activity. This result indicated that these ATPase did not a predominant target or a major cause of DOX-induced cardiotoxicity. The different result was found in the study of Bergson and Inchiosa (1985) in rabbits received 1-2 injections of DOX (4 or 8 mg/kg total) which demonstrated an increase in actomyosion ATPase activity as compared to controls and ATPase activity progressive decreased in prolonged treatment with DOX. There might be many factors influenced on activities of these ATPase, such as species of animals, dose of drug, duration of treatment etc. The

present study also demonstrated a suppression of CK activity induced by DOX. CK had a role as the catalyst of the reversible formation of adenosine triphosphate (ATP) and creatine from ADP and creatine phosphate (CP). This result showed a decrease of an energy supply of cardiac muscle after animals were treated with DOX. Myocardial infarction was also suspected (Rosalki, 1967). Loss of energy in cardiac muscle might be cardiotoxicity mediated by DOX.

In conclusion, the results of the present study indicated that acute DOX treatment depressed rate and force of isolated rat atria predominantly by decreasing the amount of Ca^{2+} in the SR. Although DOX could compete with acetylcholine at muscarinic receptor of atria, DOX also inhibited common pathway of adrenergic system. Decreased adrenergic response induced by DOX seemed to make an impact on depressed rate and force of rat atria. In subacute treatment, DOX also decreased the amount of Ca^{2+} in the SR without changing in alteration of adrenergic or cholinergic systems. DOX did not suppress the activities of myosin and actomyosin ATPase, but DOX reduced ATP supply for cardiac muscle with CK activity suppression.

This study leads to further investigate inhibitory effects of DOX on adrenergic and cholinergic receptors whether DOX acts as a competitive, noncompetitive or uncompetitive antagonist by determining ED_{50} and Pmax with varying dose of DOX.