CHAPTER II

BACKGROUND INFORMATION

Clinical Usage of DOX

The anthracycline antibiotics have been found to be one of the most active classes of antineoplastic compounds for more than three decades. One of them, doxorubicin (DOX) or adriamycin, has been used for treatment of a variety of malignancies. It has been shown to be effective in the treatment of several types of solid tumors affecting children and adults such as breast cancer, soft tissue sarcomas, endometrial cancer, osteosarcomas, tumors arising in bile ducts, esophagus and liver and non-Hodgkin's lymphoma (O'Bryan et al., 1973; Tan et al., 1973; Benjamin et al., 1974; Blum et al., 1974). The structure of DOX is shown below in Fig. 1.

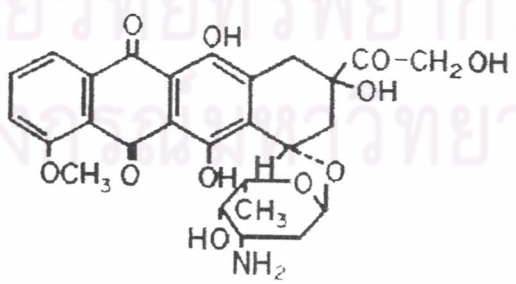

Fig. 1. Structure of DOX

Clinical Cardiotoxicity Induced by DOX

The prolonged clinical use of DOX is usually limited by the occurrence of cardiotoxicity in many patients with cancer, which has been recognized for more than 30 years (Lefrak et al., 1973). Three types of DOX-induced cardiotoxicity have been described in patients undergoing doxorubicin chemotherapy (Shan et al., 1996).

Acute and Subacute Cardiotoxicity

Acute and subacute cardiotoxicity, occurred immediately after a single dose or a course of DOX therapy, are uncommon under current treatment protocols. The clinical manifestations predominantly presented by electrocardiographic abnormalities such as nonspecific ST-T wave changes, low voltage of the QRS complex, prolongation of the QT interval, sinus tachycardia, atrial or ventricular dysrhythmias (transient arrhythmias including atrial premature contractions, ventricular premature contraction, atrial flutter or fibrillation) (Fulkerson et al., 1978; Minow et al., 1978; Von et al., 1979; Von et al., 1982; Friess et al., 1985; Steinberg et al., 1987; Shan et al., 1996). Additionally, a pericarditis-myocarditis syndrome, drug-induced coronary vasoconstriction (Bristow et al., 1978), acute failure of left ventricle (Ferrans, 1978) and sudden death attributed to arrhythmias induced by DOX (Worttman et al., 1979) had also been described. The incidence of electrocardiograhic changes associated with administration of DOX ranges from 0-41% of patients receiving the drug (Lefrak et al., 1973; Minow et al., 1978; Ali et al., 1979; Praga et al., 1979; Friess et al., 1985; Steinberg et al., 1987). These early effects are usually transient and clinically manageable by a slow 5- to 10-minute infusion, rather than rapid bolus infusion.

Chronic Cardiotoxicity

DOX-induced chronic cardiotoxicity presents with the development of a cumulative dose-related diffuse cardiomyopathy resulting in congestive heart failure or even death. This is a more common form and is the major clinical problem (Bristow et

al., 1978; Friedman et al., 1978). Chronic cardiomyopathy characteristically presented within 1 year after the completion of several doses of DOX therapy. Heart failure may occur between 0 and 231 days after the last dose of DOX (Von et al., 1979). The clinical presentation observed in these patients was similar to that seen with other diffuse cardiomyopathy. Tachycardia is the first symptom, followed by fatigue, dyspnea, nonproductive cough and peripheral edema associated with biventricular failure ([2]Bristow et al., 1978). A number of factors including total dose, schedule (Legha et al., 1982), younger age (Pratt et al., 1978), preexisting cardiac disease (Von et al., 1979), prior mediastinal radiotherapy ([3]Bristow et al., 1978; Ferran, 1978) and other cytotoxic drugs (Von et a., 1982) have been identified to increase a patient's risk for DOX-induced cardiomyopathy. The cumulative dose of the drug is the most significant risk factor for development of drug induced cardiomyopathy (Praga et al., 1979; Von et al., 1979). In adult, Von et al. (1979) reported the incidence of congestive heart failure of 0.14% at total doses of less than 400 mg/m^2. This incidence increases to 7% - 10% at a dose of 450 - 550 mg/m^2 and to 18% at a dose of 700 mg/m^2. Other prospective reports demonstrated the higher incidence (greater than 20%) of congestive heart failure at the same cumulative doses (Alexander et al., 1979). The rapid increase in clinical toxicity at doses greater than 550 mg/m^2 has made the 550-mg dose the popular empiric limiting dose for DOX-induced cardiomyopathy. Mortality directly related to DOX-induced cardiac failure is substantial; large series have reported rates of more than 20% (Praga et al., 1979; Von et al., 1979). However, Haq et al. (1985) reported up to 59% clinical recovery in patients with anthracycline-induced congestive heart failure who are treated with digoxin and diuretics.

Late-onset Cardiotoxicity

Late-onset cardiotoxicity, which occurred more than 1 year after the completion of DOX therapy particularly in children, was increasingly recognized (Leandro et al., 1994; Speyer and Wasserheit, 1998). This last type of DOX-induced cardiomyopathy caused late-onset ventricular dysfunction (Steinherz et al., 1991) and arrhythmias

(Lipshultz et al., 1991; Yeung et al., 1991). Late-onset ventricular dysfunction included decrease of fractional shortening on resting echocardiograms, increase of afterload or decrease of contractility. These abnormalities appear to be progressive and reflect future clinical decompensation (Shan et al., 1996). In survivors of childhood cancer who received prior DOX therapy at doses as low as 228 mg/m^2 up to 15 years after treatment with DOX, the incidence of cardiac abnormalities increase with time and may be as high as 65% (Lipshultz et al., 1991; Lipshultz et al., 1995). The less incidence of cardiac dysfunction (18%) of patients followed for 4 to 10 years after completion of DOX therapy was found by Steinherz et al. (1991). Additionally, late-onset ventricular arrhythmias and sudden death have been described in patients who have finished DOX therapy for more than 15 years (Steinherz et al., 1992; Steinherz et al., 1995). Simailar to the cardiomyopathy that occurs earlier, late-onset cardiomyopathy is correlated with cumulative dose of DOX, higher rates of DOX administration, and mediastinal irradiation. Studies have been performed in long-term survivors monitored for up to 20 years (Steinherz et al., 1991), and the magnitude of the problem may even be greater with increasing follow-up duration.

Preclinical Animal Models of DOX-induced Cardiotoxicity

Because of the clinical importance of DOX-induced chronic cardiotoxicity, several animal models of this syndrome, either rabbit or mouse or rat or pig or dog, for example are developed. The advantages and disadvantages of these animal models differ according to species: small animals can be used for comparative studies of anthracycline analogues and/or protectors, which may be available only in limited amounts, while larger animals can be used for investigations in which evaluations of cardiac function are to be made (Herman and Ferrans, 1998).

The first development of rat model was observed by Mettler et al. (1977). Congestive heart failure was observed in Fischer rats given weekly doses of 1 to 2 mg/kg DOX for 10 to 14 weeks. Histological examination of these animals revealed

myocyte vacuolization and degeneration, interstitial edema and mild fibrosis. Nephropathy (glomerular vacuolization, tubular damage, deposition of proteinaceous casts in the tubular lumina, and clinical and laboratory evidence of a nephrotic syndrome with hypercholesterolemia and hypertriglyceridemia) also contributed a frequent and important extracardiac effect of chronic DOX intoxication in rats. Many subsequent studies have been observed in other strains of rats. Herman et al. (1985) compared the severity of chronic DOX cardiotoxicity in adult male rats by DOX at three dose levels (0.25, 0.5, or 1.0 mg/kg/wk for 12 weeks), spontaneously hypertensive rats (SHRs) were found to be much more sensitive and suitable than other strains of rats as a small animal model of anthracycline cardiotoxicity. The chronic rat model was used continuously as a model for the evaluation of a potential cardioprotective agent, i.e. coenzyme Q_{10}, antioxidants or probucol. After rats were induced cardiomyopathy, decreased myocardial glutathione peroxidase activity, and increased levels of triglycerides, cholesterol, and high- and low-density lipoproteins by DOX at cumulative dose of 15 mg/kg, given in six equal intraperitoneal doses over a period of 2 weeks (Siveski-Iliskovic et al., 1995; Iliskovic and Singal, 1997), probucol could prevented the myocardial alterations and normalized serum levels of lipids and cholesterol. Treatment with ICRF-159 first showed to reduce toxic effect of DOX on the heart, kidney and peripheral nerves (Hu et al., 1983). Numerous investigators have made extensive use of the rat to evaluate various aspects of the mechanisms and preventive drugs for cardiotoxicity induced by DOX which will review later. With the appropriate benefit of rat model, the present study chooses this rat model for studying the subacute cardiotoxic effect of DOX.

Antitumor Effects of DOX

A number of different mechanisms have been proposed for the cytostatic and cytotoxic actions of these agents (Gewirtz, 1999). These include intercalation into DNA and/or inhibition of DNA polymerase activity (Tanaka et al., 1980; Glazer et al., 1982) with consequent inhibition of macromolecular biosynthesis (Wasserman et al., 1986;

Fritzsche and Wahnert, 1987; Munger et al., 1988), free radical formation (Sinha, 1989; Feinstein et al., 1993) with consequent induction of DNA damage (Eliot et al., 1984) or lipid peroxidation (Fukuda et al., 1992), DNA binding and alkylation (Sinha et al., 1984; Cummings et al., 1992), DNA cross-linking (Skladanowski and Konopa, 1994), interference with DNA unwinding or DNA strand separation and helicase activity (Ciarrochi et al., 1992; Tuteja et al., 1997), direct membrane effects (Tritton and Yee, 1982; Vichi et al., 1989), and the initiation of DNA damage via the inhibition of topoisomerase II (Glisson et al., 1992; Ramachandran et al., 1993). Finally, the anthracyclines have been shown to induce apoptotic cell death (Skladanowski and Konopa, 1993), although this is likely to be the final cellular response to upstream events such as inhibition of topoisomerase II.

It appears that the multiple mechanisms of action that have been ascribed to the anthracyclines may be related to the utilization of different drug concentrations under varied experimental conditions. When cells are exposed to drug concentrations in the submicromolar range, induction of cell differentiation (with prolonged exposure) and interference with DNA unwinding/DNA strand separation and DNA helicase may be evident. At drug concentrations that reflect the peak plasma concentration after bolus administration, the primary mechanism of drug action is likely to be through interaction with topoisomerase II, a conclusion that is echoed in a review by Cummings et al. (1991); it is possible, however, that the genomic site of injury plays a critical role in drug effectiveness. The interaction with the DNA-topoisomerase II complex is likely to be a primary triggering event for growth arrest and/or cell killing through a signaling pathway leading to apoptosis, at least in leukemic cells and thymocytes. At drug concentrations exceeding approximately 2-4 μM, free radical mediated toxicity and DNA cross-linking may become evident.

Mechanism of DOX Induced Cardiomyopathy

Because chronic cardiomyopathy is the most important clinical problem of DOX administration, numerous investigators try to study its mechanisms. Several mechanisms have been proposed to account for the anthracycline-induced cardiotoxic side effects. Hypotheses which are interesting and have been discussed including free radical-mediated myocardial injury (Myers et al., 1977; Doroshow, 1983; Rajagopalan et al., 1988; Singal et al., 1995), myocyte damage from Ca^{2+} overload (Singal and Pierce, 1986; Holmberg and Williams, 1990; Wang and Korth, 1995), release of vasoactive amines (Bristow et al., 1983), cellular toxicity from metabolites of DOX (Boucek et al., 1987), disturbances in myocardial adrenergic function and downregulation of myocardial beta-adrenergic receptors (Robison and Giri, 1986; Fujita et al., 1991; Tong et al., 1991). Finally, elaboration of proinflammatory cytokines, which have been consistently identified in other forms of ventricular dysfunction, may be directly relevant to anthracycline-induced cardiac injury (Ferrari et al., 1995; Shan et al., 1996). Additionally, anthracyclines cause the selective inhibition of cardiac muscle gene expression for alpha-actin, troponin, myosin light-chain 2, and the M isoform of creatine kinase in vivo (Ito et al., 1990), which may explain the myofibrillar loss associated with anthracycline-induced cardiomyopathy.

Role of Free Radicals on DOX-induced Cardiomyopathy

The cause of anthracycline-induced cardiotoxicity seems to be probably multifactorial. One of most interesting evidences points to free radical-mediated myocyte damage. DOX causes free radical formation (Olson and Mushlin, 1990) via both enzymatic and non-enzymatic mechanisms which have the potential to initiate damage to various intracellular components, including nucleic acids, lipids and proteins (Cheeseman and Slater, 1993). There is no question that under the appropriate conditions the chemistry of the anthracyclines lends itself to the generation of reactive free radicals (Bachur et al., 1978; Sinha, 1989). The quinine structure permits DOX to act

as electron acceptors in reactions mediated by oxoreductive enzymes including cytochrome P450 reductase, NADH dehydrogenase, and xanthine oxidase (Goodman and Hochstein, 1977; Pan et al., 1980; Doroshow, 1983; Graham et al., 1987). The addition of the free electron converts the quinines to semiquinone free radicals (Bachur et al., 1977; Bates and Winterbourn, 1982), which may induce free-radical injury to DNA (Eliot et al., 1984; Feinstein et al., 1993) of itself as well as after interaction with molecular oxygen to form superoxides, hydroxyl radicals, and peroxides (Feinstein et al., 1993). Increased oxygen radical activity generated through the semiquinone moiety of the DOX molecule can cause lipid peroxidation (primarily of the cell membrane) and cell injury (Rajagopalan et al., 1988); however, such lipid peroxidation would not indicate whether free radicals were being generated intracellularly or extracellularly. There is further evidence for free-radical generation mediated through the formation of complexes between DOX and iron (Zweier, 1984; Gianni et al., 1985).

The heart is particularly susceptible to free radical injury because it contains less free radical detoxifying substances (superoxide dismutase, glutathione and catalase) than do metabolic organs such as liver or kidney (Olson et al., 1981; Olson and Mushlin, 1990). Moreover, DOX is known to have a high affinity for cardiolipin, a major phospholipids component of the mitochondrial membrane in heart cells, resulting in selective accumulation of DOX inside cardiac cells (Goormaghtigh and Ruysschaert, 1984).

There are only few free radical scavengers reported to protect the heart from DOX-induced toxicity. Among them are dexrazoxane and flavonoids. Dexrazoxane (ICRF-187) is currently the only cardioprotective agent in clinical use. It has been reported to ameliorate the cardiotoxicity associated with DOX in both preclinical (Imondi et al., 1996) and clinical studies (Swain et al., 1997). Its mechanism of action appears to be the prevention of free radical formation by DOX, probably through binding of iron (Hasinoff, 1994). Flavonoids have been found to protect the heart from DOX-induced cardiotoxicity when co-administered with DOX in mice (Van Acker et al., 1996) because

of their action as both scavengers of reactive oxygen species and iron chelators (Van Acker et al., 1996). Other antioxidants, such as probucol (Siveski et al., 1994), vitamin E and *N*-acetylcysteine (Olson et al., 1981) have been reported to protect partially against DOX-induced cardiomyopathy. However, the situation is more complicated because of the existence of non-free radical damaging pathways, and the main toxic effect of DOX is the development of a cardiomyopathy long after ending of the treatment. Further the point of the free radical hypothesis, other causes of DOX-induced cardiotoxicity and their relation have been studied in many diversity.

Role of Calcium on DOX-induced Cardiomyopathy

Another popular mechanism, which has been studied in both acute and chronic cardiotoxicity is DOX-impaired Ca^{2+} homeostasis (De Beer et al., 2001). In chronic treatment with DOX, a substantial accumulation of Ca^{2+} in ventricular myocardium and Ca^{2+} inclusions in mitochondria of rabbit were demonstrated (Olson et al., 1974). DOX caused excessive rise in intracellular Ca^{2+} known as Ca^{2+} overload hypothesis (Combs et al., 1985). DOX-induced increment of the Ca^{2+} accumulation in mitochondria was generally at the expense of ATP produced by oxidative phosphorylation, thereby resulting in depletion of high-energy phosphates (Ohhara et al., 1981).

Many *in vitro* studies have reported DOX-related Ca^{2+} transport abnormalities in cardiac tissue. DOX has been reported to alter the trans-sarcolemmal Ca^{2+} influx by affecting on the Na^+/K^+-ATPase (Van Boxtel et al., 1978; Gosalvez et al., 1979; Boucek et al., 1997) and on the Na^+/Ca^{2+} exchanger (Caroni et al., 1981). Other studies have reported that DOX caused Ca^{2+} influx into the myocytes (Kusuoka et al., 1991; Wang and Korth, 1995).

Because SR plays an important role in regulating the intracellular Ca^{2+} concentration and Ca^{2+} homeostasis, a number of evidences have shown that DOX altered the function of cardiac SR (Boucek et al., 1987; Olson and Mushlin, 1990;

Ondrias et al., 1990; Boucek et al., 1993; Dodd et al., 1993). DOX has been reported to alter the Ca^{2+} release function of the SR by effects on the Ca^{2+}-ATPase and the Ca^{2+} release channel. The previous studies demonstrated effects of DOX on SR such as binding of photoaffinity labelled DOX to the Ca^{2+} release channel in fractions enriched terminal cisternae (Zorzato et al., 1986), increasing of the open probability of Ca^{2+} release channel reconstituted in lipid bilayers (Holmberg and Williams, 1990), induction of Ca^{2+} release from SR vesicles (Kim et al., 1989; Pessah et al., 1990). Wang and Korth (1995) showed that the prolongation of action potential duration due to the inhibition of I_K and the activation of SR Ca^{2+} release channel resulted in positive inotropic effect of doxorubicin in ventricular preparations isolated from guinea pig hearts. The Ca^{2+} overload hypothesis was recently supported by the observation that DOX induces down-regulation of the SR Ca^{2+}-ATPase 2 (Arai et al., 2000). The authors also provided evidence that this down-regulation was mediated by hydrogen peroxide, thereby interconnecting the free radical and this hypothesis.

Previous report of Hagane et al. (1988) determined the direct action of DOX on depression of myocardial contractility. They performed on left atrial muscle preparations of male guinea pigs. Developed tension and post-rest contraction at 10-minute intervals were examined after addition of DOX throughout 180-minute exposure to DOX. The stimulator was turned off for 30 seconds, and the first contraction observed after resumed electrical stimulation was recorded. In this study, a group of guinea pigs was injected intravenously with DOX at a dose of 2.5 mg/kg each on the first and the seventh day. After the injection completed, post-rest contraction and force-frequency relationship were examined. Direct action of DOX (100 or 200 μM) caused negative inotropic effect, prolonged time to peak twitch tension and decreased the rate of relaxation. Post-rest contraction of DOX-treated group was depressed. In atrial muscle preparations obtained from guinea pigs treated for 10 days with DOX, similar results as above were observed. These results indicated that cardiodepressant action in acute or subacute exposure to DOX was influenced with impairing the function of the cardiac SR.

However, it has been suggested that Ca^{2+} accumulation may be a manifestation rather than a cause of anthracycline cardiomyopathy. Rabkin et al. (1983) noted that the negative inotropic effects of DOX could be antagonized by increasing the Ca^{2+} concentration of the perfusate. These observations suggest that the pathogenesis of anthracycline-induced cardiotoxicity is initially associated with depletion of myocardial Ca^{2+} rather than with an excessive increase of the intracellular Ca^{2+} concentration. The decreased intracellular Ca^{2+} concentration may eventually result in the development of congestive heart failure. One of the consequences of heart failure may be accumulation of Ca^{2+} inside cardiac cells, making the increased Ca^{2+} levels observed after chronic DOX administration a consequence, rather than a cause of anthracycline-induced cardiotoxicity.

Role of Sympathetic Innervation on DOX-induced Cardiotoxicity

Adrenergic dysfunction such as downregulation of myocardial beta-adrenergic receptors (Robison and Giri, 1986; Fujita et al., 1991; Tong et al., 1991) and nonspecific blocking interaction on atrial beta-adrenergic and histaminergic receptors (Politi et al., 1985) may occur during anthracycline-induced ventricular dysfunction. Similar results were obtained in other reports (Perkins et al., 1982; Viglione et al., 1992).

The possible acute effect of DOX on cardiac beta-adrenergic mechanism was studied by Politi et al. (1985). The experiment was performed on isolated right and left guinea pig atria. Different concentration of DOX (10^{-6} – 10^{-4} M) were added to the medium 30 minutes before the concentration response curves to noradrenaline (10^{-9} – 10^{-5} g/ml) were developed. Atrial rate and inotropy of isolated right and left atria, respectively were recorded. Reserpine pretreatment was carried out by administration of 5 mg/kg of the drug 24 hours before the experiments. Only DOX at 10^{-4} M significantly reduced spontaneous atrial rate. Reserpine pretreatment did not modify the negative chronotropic activity of 10^{-4} M DOX. Thirty-minute Incubation with DOX (10^{-4} M) produced a competitive beta blocking effect, shifting to the right the concentration

response curve to noradrenaline without altering the maximal chronotropic response. Concentrations of DOX less than 10^{-4} M failed to modify the chronotropic responses to noradrenaline. The result of left atria exposed 10^{-4} M DOX for 30 minutes was similar to the right atria. However, after 60 minutes of incubation with 10^{-5} and 10^{-4} M DOX produced a shift to the right of the concentration response to noradrenaline. They also reduced the maximal chronotroic response to noradrenaline. These results demonstrated that a nonspecific interaction of DOX with cardiac beta-adrenergic receptor could be involved in the acute cardiotoxic mechanism produced by DOX. This study suggested that since DOX could be cytotoxic even without entering the cell (Triton and Yee, 1982), interaction with cell surface receptors could possibly play a role in DOX cardiotoxic mechanism.

Rasmussen et al. (1989) studied the effect of the DOX on the cardiac beta-adrenergic function *in vitro* and the development of delayed cardiotoxicity *in vivo* has been investigated in the rat. The 10^{-5} and 10^{-4} M DOX blocked the chronotropic effect of isoprenaline on isolated atria in competitive manner. Treatment with a single dose of DOX 5 mg/kg intravenously caused marked ECG changes 5 weeks after the medication. At this time no beta-blocking action was detectable in isolated atria. The results indicate that the delayed cardiotoxicity induced by DOX is not mediated by an interference with the cardiac beta-adrenoceptor function.

De Jong et al. (1990) tested the effect of DOX using isolated mouse heart muscle as in vitro model. This experiment measured the direct inotropic and chronotropic effect during 60 minutes of incubation with 10-100 μM DOX in the organ bath and determined the remaining beta-adrenergic response to l-isoprenaline after the incubation period. l-isoprenaline was used to measure the beta-adrenergic responses of spontaneously beating right and paced left atria. Both variables turned out to be equally affected. For paced left atria an IC50 (causing 50% depression of contractile force) of 35 μM was determined. Right atria stopped beating at concentrations above 50 μM. The

results indicate that DOX exert an effect not related to receptor integrity, but directly to the functionality of heart muscle.

Role of Parasympathetic Innervation on DOX-induced Cardiotoxicity

Not only adrenergic dysfunction but also cholinergic abnormality has been reported to involve in DOX-induced cardiotoxicity. DOX had been determined to act as a competitive antagonist on muscarinic receptors because DOX had been shown to inhibit negative inotropic effect of acetylcholine with shifting the concentration response curves for acetylcholine to the right in isolated guinea pig heart (Temma et al., 1992; Temma et al., 1993). Additionally, addition of atropine (10^{-6} g/ml) to the solution in the organ bath incubated with right atria was unable to modifiy the negative chronotropic activity induced by DOX (Politi et al., 1985). In the study of Viglione et al. (1992), atropine pretreatment did not affect the negative chronotropic action induced by DOX.

Chugun et al. (2001) examined the mechanisms responsible for DOX-induced reduction in the maximal negative inotropic effect of carbachol in isolated left atrial muscle preparations of pig heart. The concentration response curves for carbachol were generated after 4-hour incubation at 30 ^{o}C in the absence or presence of 30, 100 or 200 μM DOX. The 30 μM DOX caused biphasic positive inotropic effects observed at 10 and 120 minutes. The peak of the early phase was greater with 100 or 200 μM DOX. However, with 100 or 200 μM DOX, developed tension decreased during 4-hour period after the early phase peak. DOX also caused a right-ward shift of the dose-response curves for the negative inotropic effect of carbachol. The ED_{50} value, the concentration of carbachol, which caused a half maximal negative inotropic effect, was increased by DOX in a concentration-dependent manner. Moreover, the maximal negative inotropic effect observed with high concentrations of carbachol was significantly attenuated by DOX. In atrial muscle preparations exposed to 100 μM DOX for 1 hour, increase of the ED_{50} value for carbachol was similar to that observed with a 4-hour exposure, but the attenuation of the maximal negative inotropic effect of carbachol was not observed after

1-hour incubation. The effects of DOX on the muscarinic agonist were compared to those of an adenosine A_1 receptor agonist. DOX also attenuated the maximum negative inotropic effect of adenosine A_1 receptor agonist. Because muscarinic and adenosine A_1 receptor share a common signal transduction pathway in the atrial muscle, both receptors cause activation of a GTP-binding protein (Gi) to modulate ligand-regulated K^+ current. The results of this study suggested that DOX competitively antagonized binding to the muscarinic receptors and altered the pathway common to muscarinic and adenosine-induced signal transduction in rat atrial muscle.

The molecular mechanisms of anticholinergic actions of DOX were examined by electrophysiological methods in atria and myocytes isolated from guinea pig heart (Hara et al., 2000). Both carbachol and adenosine produced shortening of action potential duration in atria were measured by a microelectrode method. DOX (10-100 μM) inhibited the carbachol-induced action potential shortening in a concentration dependent manner. However, DOX did not antagonize the shortening elicited by adenosine. These results indicate that DOX produced a direct anticholinergic effect through the muscarinic receptors in atrial myocytes.

The Direct Effect of DOX on Cardiac Myofibril

Another interesting mechanism related to DOX-induced cardiomyopathy is direct interaction with the actin-myosin contractile system. Most contraction-related studies concerning the inotropic effect of DOX involved whole hearts or cardiac preparations with functionally intact membranes. However, the use of intact preparations makes it impossible to separate direct effects of DOX on the actin-myosin contractile system from effects mediated by interaction with other cellular components. Few studies focus on contractile alterations that are directly caused by interaction of DOX with the contractile apparatus. It has been reported that DOX has a high affinity for cardiac actin in vitro, probably the result of the high affinity of the daunosamine moiety in the anthracycline molecule for macromolecules (Lewis et al., 1982). Contractile changes

caused by DOX can be studied by using preparations in which both inner and outer membranes of the preparation have been permeabilized. It was shown that DOX exerts a positive inotropic effect when added to permeabilized skeletal muscle preparations of rabbits (De Beer et al., 1992). Because all membranes were permeabilized in these preparations, the contractile effect could only be caused by a direct interaction with the contractile system.

Bergson and Inchiosa (1985) studied the effects of chronic DOX treatment on cardiac actomyosin ATPase by intravenously injected to rabbits with DOX (4 mg/kg) at weekly intervals for 1-7 weeks. Body weight increase was attenuated in the treated animals; heart weight/body weight ratio was unchanged. Cellular damage was detected histologically after one dose of DOX, and was extensive after 4-5 weeks of treatment. Animals received 1-2 injections of DOX demonstrated 29% increase in actomyosion ATPase activity as compared to controls; this difference was highly significant ($p < 0.001$). Prolonged treatment with DOX resulted in progressive decrease of ATPase activity.

Watanabe and Kishikawa (1998) shown that marked necrosis and fibrosis of myocardium were observed in rats given alkaline ionized water (AKW). The activities of myosin ATPase, actomyosin ATPase and creatine kinase of rats given AKW at 15 weeks-old were compared with those of rats given tap water (TPW). The activities of myosin ATPase and actomyosin ATPase in the AKW group increased comparing with those in the TPW group. These elevated activities were caused by the degradation of myosin in the AKW group confirmed with the SDS-PAGE pattern of myosin. On the other hand, the activity of CK in the AKW group was lower than that in the TPW group. These results indicate that increases in actomyosin ATPase activity and myosin ATPase activities, with the decrease in CK activity caused myocardial necrosis and fibrosis in rats given AKW.

A few studies had been shown the effect of DOX on CK activity. DOX had been indicated to inhibit cardiac muscle gene expression for creatine kinase. Moreover, the

enzyme CK in serum had been used to investigate skeletal muscle diseases (Ebashi et al., 1959), the detection of carriers of muscular dystrophy (Aebi et al., 1962), and the diagnosis of suspected myocardial infarction (Rosalki, 1967).

Hypothesis

After the background information was reviewed, a few hypotheses which seem to have a relation to the objectives of this study are

1. An impairment of Ca^{2+} handling in the SR induced by DOX results in a decrease in rate and force of contraction of isolated rat atrial muscle preparations in acute and subacute treatment.

2. Acute and subacute treatment of DOX alter the beta-adrenergic and muscarinic receptor response to isoproterenol and acetylcholine, respectively in isolated rat atrial muscle preparations,

3. Subacute treatment with DOX causes myocardiac injury, which can be detected with changes of myocardial enzyme activities.