CHAPTER I

INTRODUCTION AND AIMS

Introduction

Doxorubicin-induced cardiotoxicity has been studied in either physiological or ultrastructural experiment. Both *in vitro* and *in vivo* experiments have been performed. The cardiotoxicity has also been reported in acute and chronic treatments.

In *in vitro* studies on intact cardiac preparations, inotropic effects of acute doxorubicin (DOX) administration have been described. Both positive inotropic effects (Van Boxtel et al., 1978; Kim et al., 1980; Temma et al., 1992; Temma et al., 1993) and negative inotropic effects (Hofling and Bolte, 1981; Politi et al., 1985; Singal and Pierce, 1986; De Jong et al., 1990; Voest et al., 1994) have been reported. Positive inotropic effects have been defined the result of direct stimulation of the cyclic AMP system or indirectly release of catecholamines, whereas negative inotropic effects have been affected 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, indirectly estimated from the decrease in the post-rest contraction. This result could be observed with either *in vitro* or *in vivo* exposure of atrial muscle to DOX.

Many studies demonstrated that DOX-induced cardiotoxicity with impacting on either adrenergic or cholinergic mechanism. These results suggest that beta-adrenergic or muscarinic receptor may be the target of DOX. Politi et al. (1985) found that acute effects of DOX produced nonspecific blocking interaction on atrial beta-adrenergic and histaminergic receptors. This result was similar to the studies reported by others (Perkins et al., 1982), but different from De Jong et al. (1990). Viglione et al. (1992)

studied the acute effects of DOX on basal rate and positive chronotropic activity induced by noradrenaline in isolated guinea-pig atria. DOX progressively depressed atrial rate after a short latency period and produced a significant reduction of the maximal chronotropic response (Emax) to noradrenaline.

Effect of DOX on cholinergic response was controversy. DOX had been determined to act as a competitive antagonist on muscarinic receptors (Temma et al., 1992; Temma et al., 1993). Hara et al. (2000) also determined that DOX produced a direct anticholinergic effect on muscarinic receptors. The result was confirmed with antagonistic action on carbachol-induced negative inotropic effect in guinea pig atria. Similarly, DOX had been shown to reverse the carbachol-induced inhibition of developed tension in isolated left atrial muscle preparation of guinea pig heart (Chugun et al., 2001). Meanwhile, *in vivo* study of Hoyano et al. (1996) had been indicated that DOX interacted with neither muscarinic receptors nor beta-adrenoceptors. However, DOX inhibited the negative chronotropic and inotropic responses to parasympathetic nerve stimulation. They suggested that DOX inhibited the negative cardiac responses to parasympathetic nerve activation due to the inhibition of acetylcholine release from nerve varicosities in the heart.

Chronic treatment of animals with DOX has been associated with contractile alterations in isolated heart preparations (De Wildt et al., 1985; Jensen, 1986; Boucek et al., 1997). These studies clearly demonstrated that DOX affected the contractile response of cardiac muscle preparations. DOX has a variety of subcellular actions inside cardiac cells (Singal et al., 1987) and many processes inside cardiac cells may be affected by DOX before the binding of Ca^{2+} to troponin C on the thin filament occurs. Additionally, DOX had been reported involving direct action on contractile apparatus with a high affinity for cardiac actin *in vitro* (Lewis et al., 1982).

Some cases of acute myocardial injury induced by DOX had been reported (Uster and Rakowsky, 1981). Bergson and Inchiosa (1985) suggested that increased

actomyosin ATPase activity seen with low doses of DOX might represent a compensatory mechanism for maintenance of contractility. Further treatment with DOX tended to progressively decrease ATPase activity. Watanabe and Kishikawa (1998) demonstrated that cause of myocardial lesions such as necrosis and fibrosis of myocardium could be detected with alteration of myosin ATPase, actomyosin ATPase and creatine kinase (CK) activities.

Many previous studies have been proposed to clarify the mechanisms of DOX-induced cardiotoxicity, but the precise mechanism of this toxicity has not been fully defined. Moreover, there is an important question whether chronic effects of DOX are similar to acute effects. There are many evidences of DOX-induced cardiotoxicity via alteration of cardiac SR function, adrenergic and cholinergic systems in acute studies, but it is lacked in the chronic investigation. Therefore, the action involving Ca^{2+} in SR or modulation of beta-adrenergic and muscarinic receptor have been proposed to be mechanisms of DOX-related cardiotoxicity in subacute or chronic treatment. Furthermore, the evidences of DOX-induced myocardial lesion in subacute treatment detected with changes in myosin ATPase, actomyosin ATPase and CK activities are also interesting in this study.

Aims

Therefore, the first objective of this present study was to determine the role of DOX on SR Ca^{2+} and changes in adrenergic or cholinergic response in Wistar rat using isolated atrial muscle preparations model in both acute and subacute (delayed cardiotoxicity) effects. The second objective was to study the enzyme activities of myosin ATPase, actomyosin ATPase and CK in subacute treatment.