Covalent Protein Binding of Reactive Adriamycin Metabolites in Rat Liver and Rat Heart Microsomes* **

M. E. Scheulen 1, H. Kappus 2, Anne Nienhaus 1, and C. G. Schmidt 1

1 Dept. of Internal Medicine (Cancer Research), West German Tumor Center, University of Essen Medical School, Hufelandstraße 55, D-4300 Essen 1, Federal Republic of Germany
2 Sect. on Pharmacology, Medical Institute of Environmental Hygiene, University of Düsseldorf, Gurlittstraße 53, D-4000 Düsseldorf 1, Federal Republic of Germany

Summary. Covalent binding of 3H-labeled adriamycin metabolites to bovine serum albumin and microsomal protein is demonstrated in an aerobic incubation system with rat liver and rat heart microsomes, respectively, using exhaustive organic solvent extraction and gel chromatography. Covalent protein binding was dependent on active microsomes, NADPH, and oxygen and was inhibited by reduced glutathione and other sulfhydryl compounds. The anthracycline moiety was spectrophotometrically evidenced in the adriamycin metabolite(s) covalently bound to protein. Thus, enzymatic activation of adriamycin in the heart with consecutive covalent protein binding of reactive adriamycin semiquinone radicals may contribute to adriamycin cardiotoxicity.

Key words: Adriamycin – Cardiotoxicity – Covalent protein binding – Metabolic activation – Rat

Introduction

The anthracycline antibiotic adriamycin is highly effective in the chemotherapy of several solid tumors and of malignant lymphomas in man (Blum and Carter 1974). Its clinical use is limited to a total dose of 550 mg/m² because of cumulative cardiotoxicity characterized by myocytolysis and congestive heart failure (Lefrak et al. 1973).

The biochemistry of the primary cardiotoxic reaction(s) of adriamycin is the matter of numerous investigations. Several biochemical mechanisms have been accused of effecting cardiotoxicity, as, e.g., destruction of DNA by single and double strand breaks (Schwartz 1975; Lown et al. 1977; Ross 1980) or by covalent DNA-binding (Sinha and Sik 1980), intercalation and inhibition of DNA- and RNA-syn-

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Offprint requests to: M. E. Scheulen, MD (address see above)
Fig. 1. Sephadex G-100 chromatography of supernatants after incubation of $^3$H-adriamycin and bovine serum albumin with rat liver microsomes with the NADPH-regenerating system

thesis (Di Marco 1975; Momparler et al. 1976; Byfield 1977), destruction of membranes by lipid peroxidation (Goodman and Hochstein 1977; Yamanaka et al. 1979) or by interaction with cardiolipin (Goormaghtigh et al. 1980), inhibition or decrease of enzymes such as metmyoglobin reductase (Taylor and Hochstein 1978), NADP-isocitrate dehydrogenase (Yasumi et al. 1980), sodium-potassium ATPase (Gosálvez et al. 1979), glutathione peroxidase (Locker et al. 1977; Burton et al. 1979; Reed and Babson 1980; Doroshow et al. 1980), ubiquinone-dependent succinoxidase and NADH-oxidase (Iwamoto et al. 1974; Bertazzoli et al. 1976; Kishi et al. 1976), uncoupling of oxidative phosphorylation (Mailer and Petering 1976) and accumulation of calcium in the myocardium (Olson et al. 1974; Olson and Capen 1978; Bühner et al. 1980; Villani et al. 1980).

Some of these biochemical effects may be closely related to the metabolic activation of adriamycin to semiquinone radicals in a NADPH-dependent, aerobic, NADPH-cytochrome P-450 reductase-catalyzed cyclic process, in which superoxide radical anions are generated (Handa and Sato 1975; Bachur et al. 1977, 1978, 1979; Doroshow and Reeves 1980).

The generation of adriamycin-semiquinone radicals, superoxide radical anions, and other subsequently formed "reactive oxygen species", such as hydroxyl radicals, singlet oxygen, and hydrogen peroxide is supposed to be the first critical toxic event in the heart, as enzymatic detoxification is less sufficient in cardiac tissue in comparison to the tissues of other organs (Doroshow et al. 1980). In addition, levels of sulfhydryl compounds are significantly depleted by adriamycin (Reed and Babson 1980; Olson et al. 1980).