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PRELIMINARY COMMUNICATIONS

BINDING OF [^{14}C]-ADRIAMYCIN TO CELLULAR MACROMOLECULES IN VIVO

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Adriamycin (ADR), an anthracycline antitumor drug, is currently one of the most promising agents for the treatment of both acute leukemia and solid tumors (1). ADR also causes cytogenetic damage of human cells as evidenced by increased chromosomal aberrations and an increase in the frequency of sister chromatid exchange (SCE) (2,3). ADR-induced chromosomal abnormalities in vivo seem to result from its ability to intercalate into DNA and its effects on nucleic acid metabolism (4,5). In this paper, we report the binding of ADR to rat liver DNA, RNA and proteins, in vivo, as a possible mechanism of ADR toxicity.

MATERIALS AND METHODS

Adriamycin·HCl (NSC 123,127) and [^{14}C]-Adriamycin·HCl (11.1 mCi/mmole) were gifts of the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Two 200 g (Charles River) male rats were used to study the binding of ADR to DNA, RNA, and proteins. Each rat was injected intraperitoneally with 0.58 mg ADR (10^{-3} M; 1.5 μCi/ml) dissolved in 1 ml of 150 mM NaCl. The rats were killed by cervical dislocation. The livers were pooled and homogenized in 10 mM sodium acetate buffer (pH 6.8) containing 0.3 M NaCl, 1% sodium dodecylsulfate (SDS), and 1% iso-amylalcohol. The homogenized livers were extracted with PhOH:$CHCl_3$ according to published methods (6). The aqueous layer containing nucleic acids was re-extracted with PhOH:$CHCl_3$ and centrifuged (6000 g, 5 min). DNA, free of RNA, was obtained by selective precipitation with isopropanol and collected by centrifugation. The isolated DNA was washed with isopropanol and dissolved in 10 mM acetate buffer, reprecipitated with isopropanol, collected, dissolved, and dialyzed against the acetate buffer.

RNA was isolated from the isopropanol solution, dissolved in the buffer, and precipitated in EtOH. RNA was collected, dissolved, and dialyzed against the buffer. Protein was precipitated by diluting the PhOH:$CHCl_3$ solution with large volumes of cold acetone, washed well with methanol, and solubilized in 1% SDS. The proteins were reprecipitated with 2% trichloroacetic acid, washed with methanol, and resolubilized in 1% SDS. Protein was assayed by the method of Sutherland et al. (7) using bovine serum albumin as the standard.

The bound radioactivity was measured in a Searle liquid scintillation counter (MARK III) using Aquasol (Universal mixture, New England Nuclear Corp., Boston, MA) and appropriate

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corrections were made for quenching. The nucleotide concentration in solutions was determined spectrophotometrically (8) using the following extinction coefficients at 260 nm: 7000 (DNA); 7200 (RNA).

RESULTS AND DISCUSSION

The binding of ADR to cellular macromolecules in liver after i.p. administration of the drug is presented in Table 1. The data show that maximum binding of ADR had occurred in 0.5 hr. Binding to DNA decreased very rapidly with time such that in 3 hr less than 10 percent of the initial binding remained. Similarly, the binding of ADR to RNA and protein also decreased with time but at a slower rate. More drug was bound to RNA at each point than DNA. The level of binding at 0.5 hr represents 1 ADR adduct/14,700 bases for DNA and 1/8,700 bases for RNA. A significant amount of the drug was also bound to proteins, confirming the earlier observations of Bachur et al. (9).

Table 1. Binding of [^{14}C]-adriamycin to cellular macromolecules in liver

Substrate	0.5 hr	1 hr	3 hr
DNA*	1.47×10^4	2.5×10^4	2.64×10^5
RNA*	8.7×10^3	1.95×10^4	4.32×10^4
Protein†	29	19	5.15

* Binding is expressed here as the molar ratio of mononucleotide unit to the drug.

† Binding to protein is defined as μmoles of the drug bound per g of protein.

Our previous in vitro studies have shown that, in the presence of a reducing agent (chemical or enzymatic), ADR binds to nucleic acids (8). The present study shows that ADR also binds to nucleic acids and proteins in vivo. This binding of ADR is time dependent. The bound drug is rapidly eliminated from liver DNA, indicating that an enzymatic repair process may be operating. Thus, the genetic damage, including increased SCE, induced by ADR may be related to binding of the drug to nucleic acids and proteins in vivo. The species and the nature of this binding to these cellular macromolecules are not known at this time. However, Moore (10) has postulated formation of the C_7-quinone methide which may function as an alkylating agent for nucleic acids and proteins.

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