AFFINITY OF ADRIAMYCIN TO PHOSPHOLIPIDS
A POSSIBLE EXPLANATION FOR CARDIAC MITOCHONDRIAL LESIONS

M.Duarte-Karim[1], J.M.Ruysschaert[2] and J.Hildebrand[1]

(1) Laboratoire de Screening Animal, Service de Médecine Interne et d'Investigation Clinique, Institut Jules Bordet, Brussels.

(2) Laboratoire de Chimie-physique des Macromolécules aux Interfaces. U.L.B., Brussels, Belgium.

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SUMMARY : Two thirds or more of adriamycin (ADM) is found in the hydrophilic phase when the drug is dissolved in the two-phase system of Folch. This distribution is changed dramatically by the presence of all the negatively charged phospholipids, which form an electrostatic complex with the drug in the lower lipophilic phase.
The molar ratio of ADM to phospholipids in the lower phase is 2 to 1 with cardiolipin, which has 2 phosphate molecules, and 1 to 1 with phosphatidic acid which has only one. ADM is recovered from lipophilic phase by acidification. No complex was obtained with sulfatides or ADM-ADN complex.
The relevance of these data to the pathogenesis of ADM induced cardiac mitochondrial lesions is discussed.

INTRODUCTION

Adriamycin (ADM) is one of the most promising new antineoplastic drugs.

Most of its side effects such as myelotoxicity, stomatitis, nausea, vomiting, and alopecia are reversible, and commonly seen with other antineoplastic drugs. Cardiac toxicity, however, is very specific and places a limit on the total dose of ADM that may be given since it is cumulative (1). So far the pathogenesis of the cardiomyopathy has not been elucidated.

The purpose of this communication is to report on the peculiar affinity of ADM for negatively charged phospholipids and to show how this property may explain the cardiac toxicity of the drug.

MATERIALS AND METHODS

The biphasic system of Folch (2) was used in this study. The proportions of chloroform, methanol and water are 3, 48, 47 by volume in the upper hydrophilic phase and 86, 14, 1 in the lower lipophilic phase.

The lipids listed in table 1 were purchased from Sigma Co (St Louis, Mo) except for galactosylceramide which was prepared from white matter and sulfatide isolated from the brain of a patient with metachromatic leucodystrophy. Adriamycin was determined by a method previously described (3). The adriamycin-DNA complex was prepared by the method of Trouet (4).

Thin-layer chromatography was performed on silica-gel G plates in chloroform, methanol, water : 65, 25, 4 (v/v/v).

RESULTS

- Interaction of adriamycin with lipids.

ADM is more readily soluble in hydrophilic solvents. When dissolved in the two-phase system of Folch, two thirds or more of the ADM are found is the upper (hydrophilic) phase. This distribution is changed dramatically by the presence of lipids. Table 1 shows that when negatively charged phospholipids were added to the biphasic system 80 % or more of ADM were found in lower (lipophilic) phase. With neutral phospholipids the percentage of adriamycin present in the lipophilic phase were equal or even lower to that of the control. However, the distribution obtained with a negatively charged glycolipid (sulfatide) was similar to that observed with the corresponding neutral galactosylceramide.

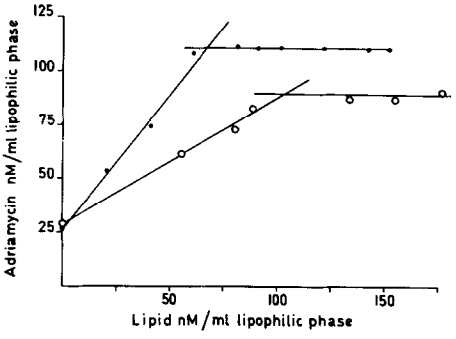
TABLE 1.

MODIFICATION OF THE DISTRIBUTION OF ADRIAMYCIN IN THE TWO-PHASE SYSTEM OF FOLCH BY VARIOUS LIPIDS

LIPIDS	PERCENTAGE OF ADRIAMYCIN IN THE LIPOPHILIC PHASE	LIPIDS CHARGE AT pH 7.2
Diphosphatidylglycerol (Cardiolipin)	97	negative
Phosphatidyl serine	94	negative
Phosphatidylinositol	93	negative
Phosphatidic acid	80	negative
Control (no lipid added)	33	—
Phosphatidylethanolamine	38	neutral
Sphingomyelin	26	neutral
Lyso Phosphatidylcholine	7	neutral
Phosphatidylcholine	5	neutral
Galactosylceramide	34	neutral
Sulfatide	37	negative
Cholesterol	10	neutral

Each assay contained in a total volume of 6 ml (2.4 ml of the upper hydrophilic phase and 3.6 ml of the lower lipophilic phase) 0.5 µmole of a given lipid and 0.1 µmole of adriamycin. Adriamycin was determined by a fluorimetric method previously described (3).

The modification of adriamycin distribution, when negatively charged phospholipids were added to the system, was studied quantitatively with cardiolipin and phosphatidic acid (Fig.1). When the amounts of adriamycin was kept constant in the biphasic system, the concentrations of the drug increased in the lower phase with increasing amounts of phospholipids. When saturating concentrations of phospholipids were reached the molar ratio of adriamycin to cardiolipin was close to 2 (1.8) and that of ADM to phosphatidic acid close to 1 (1.1).



Molar ratio between adriamycin and increasing concentrations of cardiolipin (●) or phosphatidic (○) in the lower lipophilic phase (volume 3.6 ml). The total amount for adriamycin added to the system was 200 µg, in all assays.

- Effect of pH.

By lowering the pH of the biphasic system saturated with cardiolipin, it was possible to recover increasing amounts of adriamycin in the upper phase (table II), where the drug was identified by the thin-layer co-chromatography with ADM standards.

- Interaction of acetyl-adriamycin and adriamycin-DNA complex with cardiolipin.

Less than 10 percent of acetyl-adriamycin and almost 100 % of the complex adriamycin-DNA were present in the hydrophilic phase. The addition of cardiolipin did not change significantly the distribution of these two ADM derivatives in the biphasic system.

- Stability of the adriamycin-lipid complex.

When the lower phase containing ADM and negatively charged phospholipids was dried under vacuum and chromatographed, complete dissociation of ADM and phospholipids occurred.

TABLE 2.

EFFECTS OF pH ON THE DISTRIBUTION OF ADRIAMYCIN IN THE PRESENCE OF CARDIOLIPIN

pH	ADM recovered in the hydrophilic phase (%)
7.0	3
3.7	10
2.5	50
1.3	70
0.3	Adriamycinone

Experimental conditions were as in Table 1. Modifications of pH were obtaining by adding 60 µl of HCl of increasing molarity.

However, liposomes containing ADM may be formed by sonication of phosphatidylcholine, cholesterol and cardiolipin with the drug in 0.1 M Tris-HCl buffer in 0.9 % NaCl, pH 7.2. When these liposomes were extracted with chloroform-methanol : 2.1, (v/v) and the extract partitioned according to Folch, 80 % of ADM was found in the lower phase. The analysis of the material, present in the lower phase, by thin-layer chromatography, showed two components containing ADM. The major one corresponds to native ADM; the second was more lipophilic and migrated very close to front of the chromatogram. The material corresponding to the second spot were fairly stable and could be extracted from silica gel. The structure of this ADM containing complex has not yet been elucidated, but it was established that its formation requires the presence of a hydrophilic phase.

DISCUSSION

Histologically, cardiac changes due to ADM are mainly characterized by disarray of myofilaments, vacuolization of sarcoplasmic reticulum and degeneration of mitochondria with increased electron density and myelin-like figures (5). Formation of myelin-like figures is not an infrequent subcellular lesion (6). Studies conducted in our laboratories *in vivo* (7,8), *in vitro* (9) and in a physico-chemical model (10) led to the conclusion that myelin-like figures induced in rat liver by a diazafluoranthren derivative are related to the formation of a complex between the drug and the

phospholipids. A similar mechanism was also proposed by Lüllmann and coworkers for chlorphentermine (11).

The possibility of an ADM-phospholipid complex suggested by the myelin-like figures is further supported by our data. Negatively charged phospholipids are able to displace the positively charged ADM from the hydrophilic to the lipophilic phase. Several additional observations are consistent with the formation of a complex of electrostatic nature between the positive amino-radical of ADM and negative phosphate group of phospholipids.

a) Negatively charged lipids other than phospholipids (such as sulfatide) have no effect on the distribution of ADM.

b) Negatively charged phospholipids do not appear to be able to form a complex when ADM is complexed with DNA or is acetylated on the amino function. However, because acetyl adriamycin is much more lipophilic than ADM, the interpretation of experiments with this material is difficult.

c) ADM may be recovered by acidification of the lipophilic phase.

d) The molar ratio of ADM to phospholipids is 2 to 1 with cardiolipin which has two negatively charged phosphate groups, and 1 to 1 with phosphatidic acid which has only one phosphate molecule.

These date provide a rational hypothesis for the pathogenesis of the mitochondrial lesions, one of the major and most specific sub-cellular changes characterizing adriamycin cardiotoxicity.

The rather selective toxicity of ADM for mitochondria may be due to the high concentrations of cardiolipin in mitochondria. This phospholipid is indeed an almost characteristic component of the inner membrane of mitochondria which are abundant in the cardiac muscle. In addition, the uptake of labeled ADM by mitochondria demonstrated by Rusconi (12) is in favor of our hypothesis. This theory is also consistent with the observation that the ADM-DNA complex is less cardiotoxic than ADM, and explains why acetylated ADM, which has no antimitotic activity (i.e., does not bind DNA), is not toxic for cardiac muscle. Of course, other subcellular changes such as disruption of myofilaments or vacuolization of sarcoplasmic reticulum may be due to other mechanisms like interaction with DNA-directed RNA synthesis. In addition, they may also be related the reduction of respiratory controls due to primary mitochondrial changes (13).

The interaction between ADM and phospholipids may also be involved in the effects of the drug on cell surface recently observed (14).

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