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Note

New chromatographic method for the preparation of DNA–adriamycin complexes

A. HUOT and M. PAGE
Centre de Recherches, l'Hôtel-Dieu de Québec. 11 Côte du Palais, Québec G1R 2J6 (Canada)
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The use of adriamycin as an antineoplastic agent is very often limited to patients with advanced disease because of its side effects including its cardiotoxicity[1]. Complexes of adriamycin with DNA have been prepared for injection in order to reduce this toxicity[2,3]. These complexes are active as antineoplastic agents but they still have a certain toxicity for normal cells. It is very well established that adriamycin is bound to DNA by intercalation in the flat base pairs of the double helix[4,5], but other types of binding have also been proposed[6,7].

Amberlite XAD-2 is a non-ionic resin which is used to isolate a large variety of drugs including alkaloids, barbiturates, amphetamines, phenothiazines and methadone[8]. These molecules are retained on the column by hydrophobic binding. We describe a rapid method for the preparation of adriamycin–DNA complexes using this resin, and we compare the complexes thus obtained to the ones obtained by the usual method[3,9].

MATERIALS AND METHODS

Deoxyribonucleic acid type I (calf thymus DNA) was purchased from Sigma (St. Louis, Mo., U.S.A.). Adriamycin was a gift from Adria Labs. (Toronto, Canada). Amberlite XAD-2 resin was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). All solvents and other reagents were reagent grade or of a better quality.

Preparation of Amberlite XAD-2 resin

The resin was washed extensively on a buchner funnel with methanol followed by at least 5 volumes of 0.5% sodium chloride and with 1% sodium carbonate. The resin was then washed extensively with distillated water, packed in a 1 × 10 cm column and equilibrated with 0.2 *M* Tris–HCl buffer at pH 7.5.

Chromatographic method

A 1-mg amount of adriamycin was dissolved in 1 ml of 0.2 *M* Tris–HCl buffer at pH 7.5. This solution was then added to an equivalent volume of a DNA solution (2 mg/ml) prepared in the same buffer. The mixture was stirred until the complex was dissolved. The complex was then passed through an Amberlite XAD-2 resin column (1 × 10 cm) and eluted with two column volumes of buffer. The free drug was

eluted from the resin with the following solvent: carbon tetrachloride–*tert.*-butyl alcohol–methanol–ethanolamine (1:1:1:0.5). The amount of adriamycin and DNA in the complex were measured at 480 and 260 nm, respectively[10].

Spontaneous mixture and dialysis

In order to compare the complexes prepared by the above method with the ones prepared by other procedures, complexes were prepared by the method described by Trouet *et al.*[3] utilising a 0.2 *M* Tris–HCl buffer at pH 7.5 and by dialysis[9] with a 0.01 *M* Tris–HCl, 0.01 NaCl buffer at pH 7.0. These complexes were either analysed directly or separated by chromatography as described above.

RESULTS

Table I shows that after passage on XAD-2 Amberlite resin the adriamycin–DNA ratio ($\mu g/\mu g$) was 0.088 in our assay conditions, 0.053 for the complex prepared by Trouet's method[3] and 0.026 for the complex prepared by simple dialysis. The drug–DNA ratio obtained with the last two methods correspond to the already reported ratios[3,10]. However when complexes prepared by Trouet's method were separated on the Amberlite XAD-2 column, 36% of the drug could be removed from the complex showing that the hydrophobic portion of the drug was still available for binding to the resin. This portion probably represents free drug or adriamycin bound by ionic interaction or hydrogen binding. According to the model of Pigram *et al.*[4], the hydrophobic portion of the intercalated anthracycline molecule is hidden between the base pairs of the DNA molecule, these would then be unavailable to the XAD-2 resin and the drug bound to DNA by intercalation would not be retarded by the hydrophobic resin. Most of the adriamycin bound to DNA after simple dialysis remained attached to DNA after passage on the resin, thus showing that the intercalation type of binding is favored by this procedure.

TABLE I

COMPARATIVE RATIOS OF ADRIAMYCIN–DNA

Method of purification	*μg Adriamycin / μg DNA (initial)*	*μg Adriamycin / μg DNA (after passage on Amberlite XAD-2)*	*Adriamycin bound to DNA after passage on Amberlite XAD-2 (%)*
Chromatography	—	0.088	—
Trouet *et al.*[3]	0.083	0.053	64
Dialysis	0.029	0.026	90

DISCUSSION

As shown in Table I, complexes prepared by hydrophobic chromatography have a higher adriamycin to DNA ratio. This is explainable by the higher drug–DNA ratios that could be used in the initial incubation since the loosely bound drug could be easily removed by the procedure described above. The intercalated molecules are

much more stable and susceptible to penetrate into the tumor cells by lysosomotropism as shown by Trouet *et al.*[3].

The described procedure being rapid offers another advantage over the dialysis method since adriamycin loses its activity within 48 h in aqueous solutions.

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