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Short communication

Poly(I) · Poly(C), a potential drug carrier for the antitumor agent mitoxantrone: in vitro drug binding study

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Summary. Coupling of mitoxantrone, a new antitumor agent, to a macromolecular carrier system may improve the drug's selectivity of action and pharmacokinetic properties. We have studied in vitro binding of mitoxantrone to poly(I)·poly(C), a macromolecular, double-stranded homoribopolymer, by equilibrium dialysis and high-performance liquid chromatography (HPLC).

Results showed high binding affinity for mitoxantrone to poly(I)·poly(C) ($K_d = 1.05 \cdot 10^{-6} M$), the calculated number of mitoxantrone-binding sites is 60 per molecule poly(I)·poly(C). In view of the good tolerance in clinical studies, poly(I)·poly(C) may thus be a useful drug carrier for mitoxantrone. A mitoxantrone:poly(I)·poly(C) ratio of 1:30 (w/w) is recommended for therapeutic studies.

Introduction

Coupling of antitumor drugs to macromolecular drug carrier systems may increase the low therapeutic index of these drugs. In vitro and animal studies showed a more selective cytotoxic effect on tumour cells of 5-fluorouracil and doxorubicin bound to nanoparticles [8] and of adriamycin bound to DNA [2, 3]. This enhanced selectivity may be due to an increased endocytotic uptake of the bound drug by malignant as compared to normal cells [9, 12]. Macromolecular binding may also improve pharmacokinetic parameters, e.g., reducing systemic absorption of cytostatics injected into the peritoneal or pleural cavity.

Poly(I).poly(C), a double-stranded synthetic homoribopolymer, is a potentially useful macromolecular carrier for intercalating cytostatics: clinically, it was tolerated without severe side effects [1]; in vitro, pinocytotic cellular uptake of poly(I).poly(C)-dye complexes has been demonstrated [5]. Mitoxantrone is a recent anthracenedione derivative with favorable clinical results [10]; is appears to act as an intercalating agent [6, 7].

We have studied in vitro binding of mitoxantrone to poly(I).poly(C) as a preliminary for clinical studies with mitoxantrone – poly(I).poly(C) complexes.

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Materials and methods

Materials. Mitoxantrone (Novantron®), 2 mg/ml, was purchased from Cyanamid, Wolfratshausen, FRG. Lyophilized poly(I).poly(C) (molecular weight 350000) was obtained from Böhringer Mannheim GmbH, Mannheim, FRG. Tubes for equilibrium dialysis (Thomapor; molecular weight cut-off 10000) were from Reichelt Chemietechnik GmbH, Heidelberg, FRG.

Equilibrium dialysis. Binding of mitoxantrone to poly(I).poly(C) was studied by equilibrium dialysis: Appropriate amounts of poly(I).poly(C) were dissolved in phosphate buffered saline (PBS; pH 7.4) to yield concentrations of 100, 30, 10, 3, and 1 µg/ml. Next, 2-ml volumes of these solutions were placed in dialysis bags. The bags were knotted and placed into plastic containers filled with 100 ml PBS which contained mitoxantrone at concentrations indicated in Fig. 1. The containers were gently shaken for 24 h at room temperature. Thereafter samples from inside and outside the dialysis bags were retrieved and mitoxantrone concentration was analyzed on the same day.

Mitoxantrone analysis. Mitoxantrone concentration was determined by high-performance liquid chromatography

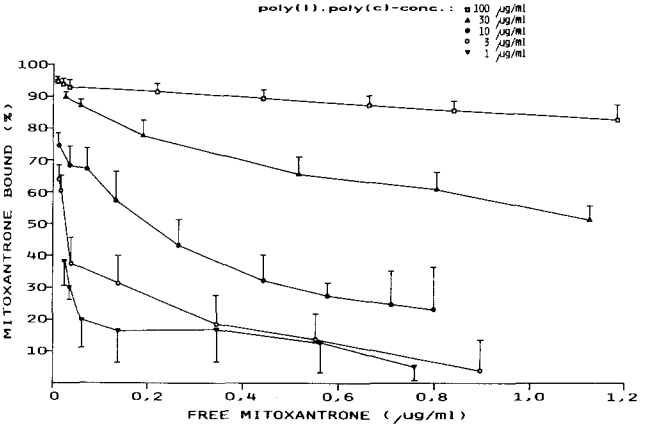

Fig. 1. Mitoxantrone binding to various concentrations of poly(I).poly(C). Calculation of percentage bound is described in Methods. Results are means ±1 SD of duplicates of three experiments. Concentrations of poly(I).poly(C): □ 100 µg/ml; ◀30 µg/ml; ● 10 µg/ml; ○ 3 µg/ml; ▼ 1 µg/ml

(HPLC) according to a method described in detail by Ehninger et al. [4]. The chromatographic system consisted of a Hewlett Packard (HP) 1090 A with a fixed wavelenght spectrophotometer and an HP 85 B processor. Separation was obtained with a Waters Associates μ Bondapak C18 column (30 cm × 3.9 mm ID; 10 μm particle size). The guard column was packed with CO:Pell ODS (Whatman). The system for mitoxantrone resolution consisted of acetonitrile:water (27:73) containing 5 m*M* 1-pentane-sulfonic acid (PIC B-5, Waters Associates). The flow rate was maintained at 1 ml/min. Mitoxantrone was detected at 254 nm. Next, 20–80 μl of the untreated aqueous samples were injected into the HPLC system. Preliminary experiments had shown that the presence of poly(I).poly(C) at concentrations used in this study had no effect on mitoxantrone recovery in the HPLC assay. Samples from inside the dialysis bags were therefore treated identically to outside samples. The detection limit of the assay was 10 ng/ml. The coefficient of variance at 100 ng/ml was 6.5%.

Calculations. Percentage of mitoxantrone bound to poly(I).poly(C) were calculated for each concentration of poly(I).poly(C) and mitoxantrone (M) as follows:

$$\% \text{ of M bound} = \frac{\text{conc}_M \text{ inside} - \text{conc}_M \text{ outside}}{\text{conc}_M \text{ inside}} \times 100$$

The number of mitoxantrone binding sites per molecule poly(I).poly(C) was calculated according to Scatchard [11].

Results

Poly(I).poly(C) precipitated upon interaction with mitoxantrone at high concentrations; therefore, all samples were centrifuged at 3000 rpm for 10 min and only samples having no visible precipitate were analyzed. Thus, further extension of the binding curves (Fig. 1) to higher concentrations of mitoxantrone and/or poly(I).poly(C) was not possible.

Figure 1 shows the percentage of mitoxantrone bound versus free mitoxantrone concentration. As expected, the percentage of drug bound increased with the amount of poly(I).poly(C) available. A Scatchard analysis using data from the three highest poly(I).poly(C) concentrations showed 60 ± 7 mitoxantrone binding sites per molecule poly(I).poly(C). The dissociation constant was $K_d = 1.05 \pm 0.18 \times 10^{-6} M$. At the lower poly(I).poly(C) concentrations the binding plot was nonlinear. This is most likely the result of electrostatic interactions between the drug and the exterior of the ribopolymer [7]. Electrostatic interactions may also be involved in the precipitation observed at higher mitoxantrone/poly(I).poly(C) concentration ratios [7].

Discussion

These results indicate that mitoxantrone strongly binds to poly(I).poly(C); the dissociation constant was in the same order of magnitude as that for native DNA [7]. According to the Scatchard analysis, 1 mol of poly(I).poly(C) (molecular weight 350000) binds approximately 60 mol mitoxantrone (molecular weight 444.5). Thus 1 mg poly(I).poly(C) would bind 0.08 mg mitoxantrone at full saturation of the binding sites. A mitoxantrone:poly(I).poly(C) ratio of approximately 1:30 (w/w) should, however, not be exceeded for clinical application if > 50% of mitoxantrone is to be bound. Precipitation of poly(I).poly(C) – mitoxantrone mixtures at concentrations > 1.2 μg/ml mitoxantrone limits its use for intravenous therapy because excessive volumes of fluid would be necessary to deliver therapeutic mitoxantrone doses (8–15 mg/m^2). However, precipitation would not be a problem when poly(I).poly(C) – mitoxantrone is administered into a body compartment where embolization is not a risk. We have administered up to 45 mg mitoxantrone and 170 mg poly(I).poly(C) in 1–2 l saline intraperitoneally to patients. Although there was a visible precipitate, no adverse effects were observed (unpublished results).

In conclusion, poly(I).poly(C) is a potentially useful drug carrier for mitoxantrone, especially for intrapleural or intraperitoneal application, where the macromolecular binding may reduce systemic absorption. Clinical studies seem warranted.

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