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Polycationic polymers and drugs: Investigations into interactions between acyclovir and polymers

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ABSTRACT

In the development of drug formulations interactions between contents may play an important role. It was the aim of our present studies to investigate into drug-polymer interactions. Interaction of the antiviral drug acyclovir with polyethylenimines, polyvinylamines and the non-ionic PVP was investigated using a modified equilibrium dialysis. The membrane was permeable only to free acyclovir, while polymers and acyclovir-polymer-associates did not pass through. Significant amounts of acyclovir were bound with the polyethylenimines. The formation of associates consisting of acyclovir and PVP or polyvinylamine could not be demonstrated. In solutions of acyclovir and polyethylenimine (Mr=25000) the amount of bound drug is increased with increasing concentration of acyclovir. Between 7.9 µg and 31.7 µg take part in the formation of associates. Differences in the osmotic pressure of the solutions do not play an important role in the permeation of acyclovir. In solutions containing acyclovir and high molecular weight polyethylenimine (Mr=750000) the bound amount of drug increases with increasing acyclovir concentration up to c_0=400µg/100 ml. A further increase does not change the amount of bound drug significantly. Possibly, the binding capacity is reached. Molecular modeling investigations were performed. According to the calculations, about 85% of the interactions can be attributed to electrostatic interactions.

KEYWORDS

Acyclovir;
Drug-polymer interactions;
Equilibrium dialysis;
Molecular modeling;
Polyethylenimine;
Polyvinylamine;
Polyvinylpyrrolidone.

INTRODUCTION

Polyethylenimines (PEI) are cationic polymers, which can be used in very different fields[1-9]. The polymer can form complexes with different substances, like phenols, azo-dyes and sodium dodecyl sulphate[10-12]. The binding ability is also used in cell-experiments: PEI is tested as a transfection reagent[2,13-17]. To get information about the binding of polymers and their binding partners different physicochemical methods can be used. Methods like IR and DSC give information about interactions in the solid state[9,18-21]. If polymer solutions shall be examined, equilibrium dialysis is an established method to investigate into interactions between polymers and low molecular weight substances[11,12,22-27]. The equilibrium dialysis systems consist of two cells, which are separated by a semipermeable membrane. Only low-molecular weight

substances can pass this barrier. To start the experiment, solutions of the polymer and the possible binding partner are placed in the dialysis cells. In equilibrium status, changes in the concentration of the low-molecular weight substances in one or both of the dialysis cells can give information about polymer binding.

In our present investigations, the non-covalent bonding between drugs and polymers like polyethylenimine is investigated. A non-covalent bonding of drugs to polymers may result in enhanced solubility of the drug[18,19,22,28,29]. Therefore, it is important to get more information about polymer-drug interactions in the field of dosage form development. The antiviral acyclovir was chosen as model drug, polyvinylpyrrolidone, polyvinylamine and polyethylenimines of different molecular weight were chosen as polymers. Information about interaction between acyclovir and the polymers should be gained from equilibrium dialysis experiments. Further, the osmotic pressure of the solutions should be measured. Molecular modeling studies should give information about possible interaction mechanisms.

MATERIALS AND METHODS

Acyclovir was purchased from Fährhaus Pharma (Hamburg, Germany). PVP K30 was obtained from Sigma-Aldrich (Steinheim, Germany). Polyethylenimine HF, Polyethylenimine P and Polyvinylamine were purchased from BASF (Ludwigshafen, Germany). The equilibrium dialysis experiments were performed with modified Transwell™ systems (Corning Inc., CA Acton, USA). The membrane of the Transwell™ systems was substituted by a dialysis membrane (ZelluTrans® Dialysierschlauch, Art. No E658.1, Roth, Karlsruhe, Germany). The membrane has a molecular weight cut off (MWCO) of 4000-6000. Therefore, the polymers could not pass the membrane. Acyclovir could pass the membrane, as demonstrated in experiments. Aqueous solutions of acyclovir with the concentrations 0.1 mg/100 ml, 0.2 mg/100 ml, 0.3 mg/100 ml,0.4 mg/100 ml and 0.6 mg/100 ml were prepared. Further, aqueous solutions with analogue acyclovir concentrations and polymers were prepared: Polyvinylamine HF had a concentration of 0.222 g/100 ml, polyethylenimine P of 0.33 g/100 ml. Solutions containing 0.444 g PVP K30 were manufactured. In case of Polyvinylamine, solutions of 0.4 g/100 ml were used. Before the dialysis experiments were started, the membranes had been saturated with acyclovir solutions of the corresponding drug concentration. 2.0 ml of the polymer-drug solutions were filled in the bottom of the modified Transwell™ systems, 2.0 ml of the acyclovir solutions with the corresponding drug concentration in the top. The Transwell™ systems were stored at room temperature for 25 h. It had been shown in preliminary tests that the drug transport between the two solutions has reached equilibrium after this time. The amount of acyclovir was determined using high performance liquid chromatography. The HPLC apparatus consisted of a L-2130 pump and an UV-detector L2400 (VWR™ International, Darmstadt, Germany). A RP-18 column (Hibar® RT 125-4, filled with LiChrosorb RP-18, 5 µm, Merck, Darmstadt, Germany) was used as stationary phase. The mobile phase consisted of methanol and distilled water in ratio 2+8. The flow rate was 1 ml/min. The injection volume was 20 µl. A wave length of 256 nm was chosen for detection of acyclovir. The peak area under the curve was calculated and related to an extern calibration standard. An acyclovir USP reference standard (USPC, Rockville MD, USA) was used in the analytical procedure.

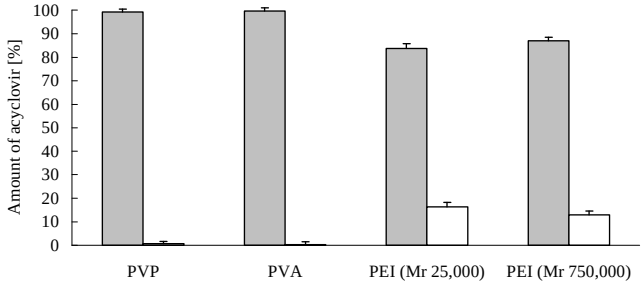
To investigate into the osmotic pressure of drug and polymer solutions, solutions of acyclovir (0.1 mg/100 ml and 0.2 mg/100 ml), polyvinylamine HF (0.222 g/100 ml), polyethylenimine P (0.33 g/100 ml) and PVP (0.444 g) were prepared and measured using a semi-micro-osmometer (A0300, Knaur, Bad-Homburg, Germany). Preliminary IR studies were performed using a FT/IR-480 Plus Spectrometer (Jasco, Groß-Umstadt, Germany) (acknowledgments to Dr. D. Schepmann) and a IR Spectrometer 1320 (Perkin-Elmer, Überlingen, Germany).

Molecular modeling investigations were performed using the program HyperChem™ (Release 7.51, HyperCube, Inc. USA). To get information about possible complexes between polymer and drug and about interactions between acyclovir and a PEI fragment, molecular dynamic and molecular mechanic calculations were performed using the AMBER force field. Water molecules were included in the molecular dynamic simulations. The kind and amount of interaction in different complexes was calculated. During molecular

dynamic simulations, the distance between interacting parts of the molecules was monitored. Detailed information about the calculations is given in the chapter "Supplementary information".

RESULTS AND DISCUSSION

The interactions between the antiviral drug acyclovir and various polymers were investigated. Equilibrium dialysis experiments were performed with solutions of acyclovir and PVP, polyethylenimines with a molecular weight of 750000 and 25000 and polyvinylamine. The molar ratio of the polymers and acyclovir was 20:1 except for high molecular weight polyethylenimine and acyclovir. In this case, the substances were used in equimolar amounts. Interactions were observed between the drug and the polyethylenimines (Figure 1).



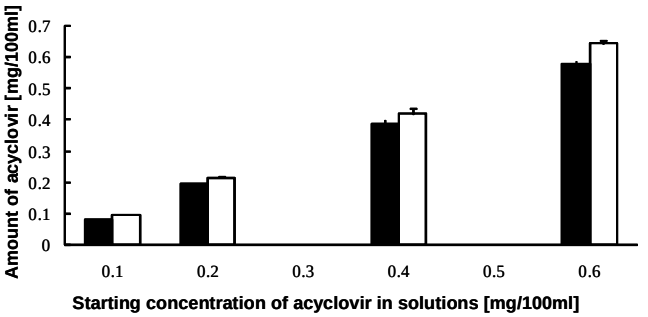
■ amount of acyclovir [%] in the acyclovir solution
□ amount of acyclovir [%] which has permeated into the acyclovir-polymer-solution

Figure 1 : Dialysis of acyclovir between an acyclovir solution (c_0=0.1 µg/100 ml) and an acyclovir-polymer solution after 25 h:

In solutions containing acyclovir and polyvinylamine or PVP, no interactions could be detected in dialysis experiments. To determine if interaction between acyclovir and polyvinylamine occur at increased drug concentrations, the amount of acyclovir was doubled, quadrupled and sextupled. No significant amounts of acyclovir were bound with polyvinylamine. Likewise, in PVP-acyclovir solutions with a molar ratio up to 5:1, no interactions were detected.

To get information if the permeation of acyclovir was influenced by differences in the osmotic pressure of drug and polymer solutions, the osmotic pressures of the solutions were measured using a semi-micro osmometer. No differences in the osmotic pressure of the pure and mixed solutions could be measured, the concentrations of drug and polymers was very low.

The dependency of the drug concentration on the acyclovir-polyethylenimine interaction was investigated. Polyethylenimine with a molecular weight of 25000 was used. Dialysis experiments were performed using acyclovir solutions and acyclovir-polymer solutions with drug concentrations between 100 and 600 µg/100 ml. With increasing amount of acyclovir the bound amount of acyclovir is increased (Figure 2). 7.9, 9.9, 15.1 µg or 31.7 µg acyclovir take part in interactions.

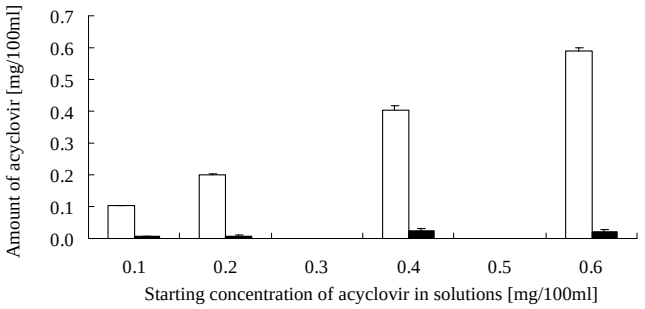


■ amount of acyclovir in the acyclovir solution, end of dialysis
□ amount of acyclovir in the acyclovir-PEI solution, end of dialysis

Figure 2 : Dialysis of acyclovir between solutions containing acyclovir (0.1 µg/100 ml, 0.2 µg/100 ml, 0.4 µg/100 ml, 0.6 µg/100 ml) and solutions containing low polyethylenimine (Mr: 25,000) (0.222 g/100 ml) and acyclovir of the accordant concentration

Further investigations into the interactions between acyclovir and high molecular weight polyethylenimine were performed. The amount of acyclovir in the drug solution and in the drug-polymer solution was varied between 100 µg and 600 µg/100 ml. Dialysis experiments were performed. The bound amount of acyclovir increased with increasing acyclovir concentration up to 400 µg/100 ml. At a starting concentration of 600 µg/100 ml acyclovir, no further increase of the bound amount was observed (Figure 3).

To get information about possible interactions between polyethylenimine and acyclovir, preliminary IR studies and molecular modeling investigations were performed. In physical mixtures of PEI with a molecular weight of 25,000 and acyclovir slight changes in the spectra were observed. Changes in the wave number in the area of 1590 nm indicate that the imidazole cycle of acyclovir could be affected by the polymer. Further, the hydroxyl group seems to be affected. Molecular modeling calculations of complexes with acyclovir and



□ **amount of acyclovir in the acyclovir-PEI solution, end of dialysis**

■ **amount of acyclovir associated with polymer, end of dialysis**

Figure 3 : Dialysis of acyclovir between solutions containing acyclovir (0.1 µg/100 ml, 0.2 µg/100 ml, 0.4 µg/100 ml, 0.6 µg/100 ml) and solutions containing high molecular weight polyethylenimine 0.3 g/100 ml) and acyclovir of the accordant concentration

a PEI fragment were performed to get more information about possible interactions. Six associates with calculated low total energy and with high absolute values of interaction energy were chosen for detailed investigations. According to the calculations, between 75 % and 95 % of the interaction energy can be attributed to electrostatic interactions. Hydrogen bonding is found in each of the six associates. The imidazole cycle is a preferred partner for H-bonding. Interactions between the acyclovir-oxygen and activated protons of the polymer are also observed. The amount of hydrogen bonding on the calculated energy, which is attributed to drug-polymer interaction, varies between 1.9 % and 4.5 %.

Molecular dynamic studies with the above mentioned drug-PEI complexes in a simulated water box were performed. According to the calculations, the complexes are stable in the time scale of the simulation. Interactions between acyclovir and the polyethylenimine fragment in simulated aqueous environment were observed. Since the preliminary IR studies gave hints that the imidazole ring might take part in interactions, the focus of the investigations was set on this cycle. In one complex, the imidazole ring of acyclovir was in close contact to amino groups of the polymer in 96.9 % of the analyzed geometries (0.17 – 0.29 pm), the ether oxygen in 100 %. The drug was located between two branches of the polymer fragment. In the molecular dynamics simulation of the second complex, acyclovir was located at the outer sphere of the polymer fragment. While the imidazole ring stayed in close to looser contact with polyethylenimine (18.8 %: 0.17 – 0.29 pm, 67.2 %: 0.3-0.39 pm), the oxygen was located in greater distance to the polymer most of the time (94 %: >0.4 pm).

Equilibrium dialysis experiments were performed using a membrane which is not permeable for the macromolecules. Therefore, the different concentration of acyclovir in the polyethylenimine solutions and a polymer-free drug solution is a hint for physicochemical interactions between acyclovir and the polymers. According to molecular modeling calculations, electrostatic interactions between acyclovir and polyethylenimine play an important role in the formation of associates. Acyclovir does not form associates with every cationic polymer, as demonstrated in experiments with polyvinylamine-acyclovir solutions. Possible reasons may be different basicity and different geometry: the polyvinylamine chosen for our experiments is a linear polymer, the chosen polyethylenimines are branched. According to the molecular modeling studies, acyclovir can be located between different braches of PEI and can take part in interactions.

In solutions of acyclovir and polyethylenimine with a molecular weight of 25000 the binding capacity of the polymer is not reached at a polymer-drug molar ratio of about 3:1. The binding capacity of the polymer increases with increasing molecular weight. At low acyclovir starting concentrations, the molar binding capacity of the high molecular weight polyethylenimine is increased by the factor 17, compared to the binding capacity of polyethylenimine with a molecular weight of 25000. The binding capacity does not increase analogue to the molecular weight, probably because polyethylenimines have branched structures.

A distribution of a low-molecular weight substance like acyclovir between two dialysis chambers may be influenced by significant differences in the osmotic pressure of the two test solutions. According to our studies, differences in the osmotic pressure of drug and polymer solutions do not play an important role in the permeation of acyclovir through the dialysis membrane. The chosen molar concentrations of acyclovir and of the polyethylenimines is very low. No differences in the osmotic pressure of the pure and mixed solutions could be measured in preliminary tests.

Molecular modeling calculations were performed with polyethylenimine fragments. The molecular weight of the fragments is about 1470. The fragments are too small to draw conclusions about a possible degree of drug loading of polyethylenimines. The calculations were performed using the AMBER force field. A hydrogen bonding term is integrated in the force field. Therefore, it was possible to gain information about hydrogen bonding.

SUPPLEMENT INFORMATION AVAILABLE STATEMENT

Detailed information about building up the molecules and complexes and about settings and procedure of geometry optimization and molecular dynamic simulations.

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