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Adsorption of hematoporphyrin onto polyalkylcyanoacrylate nanoparticles: carrier capacity and drug release

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Summary

Experimental conditions for the formulation of hematoporphyrin loaded nanoparticles are presented. The adsorption was performed by incubation of unloaded nanoparticles with hematoporphyrin and was found to be dependent on the pH of the incubation medium. The maximum loading was achieved at pH 5.9 with a carrier capacity of about 30 μg HP/mg polyisobutylcyanoacrylate nanoparticles. The pH effect was correlated to the degree of ionization of hematoporphyrin. A rapid drug release from nanoparticles was, however, observed following dilution in phosphate buffer at pH 5.9 and was more important at pH 7.4. This fast release reflected an equilibrium shift between adsorbed drug and free drug. The addition of esterases to nanoparticles suspension modified this equilibrium by solubilization of the polymeric sites of drug adsorption and led to an increase in drug release. The potential of photosensitizer loaded nanoparticles in photodynamic therapy is briefly discussed.

Introduction

Porphyrins are macromolecules with inherent photosensitizing properties which are highly taken up and retained in tumor tissues. These properties have led to their use in photodynamic therapy (Kessel, 1990). Local illumination of malignant tumors with red light in a few days following intravenous administration of porphyrins results in the production of singlet oxygen with damage to biomolecules and subsequent tumor cell death. However, some retention of porphyrins in the skin induces cutaneous photosensitivity in patients who have to avoid bright light for 1 month or more after phototreatment. To prevent this unpleasant side effect and to improve the efficiency of photodynamic therapy, there is a need to target the photosensitizer more accurately to cancer cells. One way to satisfy this is to use colloidal drug delivery systems like liposomes (Jori et al., 1983). However, problems associated with stability of liposomes in biological fluids and during storage have led to the development of polymeric drug

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Abbreviations: PIBCA, polyisobutylcyanoacrylate; PIHCA, polyisohexylcyanoacrylate; HP, hematoporphyrin.

carriers. Among them, polyalkylcyanoacrylate nanoparticles are obtained by emulsion polymerisation in an aqueous phase at low pH in the presence of a stabilizing agent (Couvreur et al., 1979; 1982a). Owing to their polymeric nature, these small biodegradable particles (diameter of 50–250 nm) are able to adsorb a variety of drugs in a stable and reproducible way. Several studies conducted on tumor models in rodents have shown that the association of cytostatic drugs to these nanoparticles modifies their tissue distribution and improves their antitumoral activity while reducing their systemic toxicity (Brasseur et al., 1980; Couvreur et al., 1982b; Kreuter and Hartman, 1983; Chiannilkulchai et al., 1990; Verdun et al., 1990).

This paper describes the technique of attachment of hematoporphyrin (HP) to polyisobutylcyanoacrylate (PIBCA) and polyisohexylcyanoacrylate (PIHCA) nanoparticles as well as the factors affecting the release of the drug from these polymeric carriers.

Materials and Methods

Materials

Isobutylcyanoacrylate monomer (IBCA) and esterases from hog liver were purchased from Sigma (Coger, Paris, France). Isohexylcyanoacrylate monomer (IHCA) was supplied by Sopar (Brussels, Belgium). Dextran 70 was obtained from Fluka (Buchs, Switzerland). Hematoporphyrin (HP, structure shown in Fig. 1) was purified from commercial hematoporphyrin (Roussel, France) according to published method (Vever-Bizet et al., 1984). Stock solutions were prepared by dissolving HP in 0.3 N NaOH followed by addition of 0.3 N HCl to reach neutrality and isotonicity. The desired pH and concentration were obtained by dilution with an isotonic phosphate buffer adjusted to this pH.

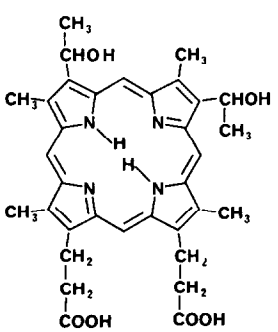

Fig. 1. Structure of hematoporphyrin (HP).

Nanoparticle preparation

During nanoparticle formation, the pH has to be lowered to below 3 to slow down the anionic polymerisation reaction. Since HP is poorly soluble in the pH range from 2 to 5.8, this drug was added to preformed nanoparticles in a buffer solution adjusted to neutral pH values.

Practically, unloaded nanoparticles were prepared under mechanical stirring by adding 50 μl of IBCA monomer to 5 ml of hydrochloric acid (0.001 M) containing 1% dextran 70 and 5% glucose. After complete polymerisation (2 h for IBCA, 6 h for IHCA), the nanoparticle suspension was neutralized by addition of 2 ml of an isotonic phosphate-buffered solution ($NaH_2PO_4 \cdot H_2O$, 0.155 M; Na_2HPO_4, 0.0119 M; pH range 5.9–7.4) containing hematoporphyrin at different concentrations. The mixture was left overnight at 20 °C before determining the amount of drug adsorbed. The size of the particles was determined by means of a laser light scattering method (Coulter N4MD, Coulter electronics Inc., Hialeah, U.S.A.).

Determination of drug content

HP concentration was determined on the whole nanoparticle suspension (drug initially added) as well as on the supernatant (free drug) and on the nanoparticle sediment (adsorbed drug) obtained after ultracentrifugation of the preparation at $100\,000 \times g$ for 1 h. Direct spectrophotometric analysis was performed at 400 nm on samples diluted in sodium hydroxide solution 20 mM containing 2% sodium dodecyl sulfate. This solvent allows for the chemical degradation of the nanoparticles as well as the complete monomerisation of hematoporphyrin (molar extinction coefficient, $\epsilon = 2 \times 10^5\ M^{-1}\,cm^{-1}$ at 400 nm).

Drug release from nanoparticles

HP-loaded PIBCA nanoparticles used for drug release studies were obtained by incubation in phosphate-buffered medium (pH 5.9) of preformed nanoparticles together with an initial concentration of 180 μg HP/ml. Under these conditions, a large amount of drug (about 80%) was associated with nanoparticles. Free HP used as a control was found to be very stable in the same conditions of incubation. The release of HP from nanoparticles was performed at 37°C under mechanical stirring after a 10-fold dilution of the nanoparticle suspension in an isotonic phosphate-buffered solution (pH 5.9 or 7.4) in the presence or absence of esterases (100 μg/ml). At this dilution, the concentration of HP was well below 10% of its solubility in buffer medium at pH 5.9, and this corresponded also to sink conditions. Sodium azide (100 ppm) was added as antimicrobiological agent to the incubation medium. At different time intervals, samples were taken out to measure nanoparticle size, turbidity and drug-release. Turbidity analysis was performed by direct spectrophotometric analysis of the sample at 470 nm. The percentage of drug released from nanoparticles was determined after ultracentrifugation of the incubation medium (100 000 × *g*, 1 h) and spectrophotometric analysis at 400 nm of HP content in both the supernatant and the sediment as described above.

Results and Discussion

Particle size analysis

The average diameter of unloaded nanoparticles was determined to be 147 nm with a standard deviation of 37 nm for PIBCA nanoparticles. The size remained unchanged when nanoparticles were loaded with increasing concentrations of HP (25 to 750 μg/ml) at different pH (5.9–7.4). PIHCA nanoparticles behave in the same way.

Adsorption of HP to nanoparticles

Effect of pH The effect of the pH of the incubation medium on the association of HP to preformed PIBCA nanoparticles is presented in Fig. 2. The adsorption efficiency of HP onto

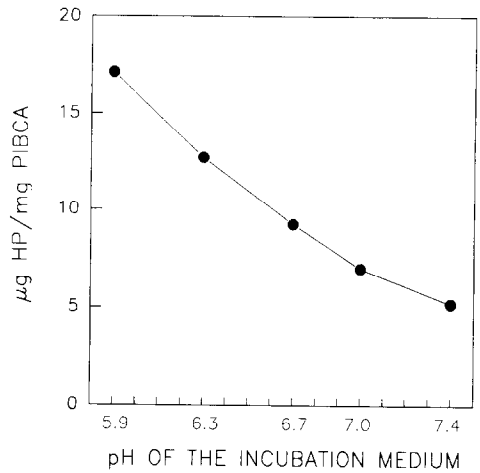

Fig. 2. Adsorption of hematoporphyrin (μg HP/mg PIBCA) onto PIBCA nanoparticles as a function of the pH of the incubation medium (initial concentration of HP: 180 μg/ml).

nanoparticles was found to decrease drastically as the pH of the incubation medium increased from 5.9 to 7.4. As an example, when the initial concentration of HP was 180 μg/ml, 83% of the drug was associated with PIBCA nanoparticles at pH 5.9. This percentage was reduced to 26% at pH 7.4. The same observation was done with PIHCA nanoparticles (data not shown). These results can be explained by the fact that the degree of ionization of the drug affects its adsorption onto nanoparticles, with a maximum association when the drug is in its non-ionized form (Couvreur et al., 1979). In the case of hematoporphyrin, successive protonation of the inner nitrogen of the cycle and successive deprotonation of propionic groups occur at the following pK_a values: 4.7, 2.9, 5.0 and 5.5, respectively (Brault et al., 1986; Brault, 1990). As a consequence, HP is less ionized and therefore more extensively adsorbed to nanoparticles at pH 5.9 than at higher pH values. This pH effect has also been described by Brault and collaborators (1986) when studying the incorporation of HP in egg phosphatidylcholine vesicles. Moreover, the negatively charged forms of HP which are predominant at pH 7.4 are less susceptible to associate to slightly negatively charged polyalkylcyanoacrylate nanoparticles (Kreuter, 1983).

Effect of HP concentration The influence of the concentration of HP in the incubation medium

(pH 6.2) on its adsorption onto PIBCA nanoparticles is shown in Fig. 3. Even if the amount of drug adsorbed per mg of polymer increased with drug concentration (up to 750 μg/ml) in the incubation medium, the percentage of associated drug dropped dramatically from 83% at 25 μg HP/ml to 30% at 750 μg HP/ml. The same conclusion was drawn for PIHCA nanoparticles, with a somewhat greater amount of drug associated to the polymer (data not shown).

By comparison with the adsorption of a gas on a solid as a function of the equilibrium pressure at constant temperature, the relationship between the amount of drug adsorbed onto nanoparticles and the concentration of drug at equilibrium yields an adsorption isotherm (Martin et al., 1983). Our data seem to fit best with the Langmuirian isotherm model (correlation coefficient 0.995). This would suggest that the drug is adsorbed onto nanoparticles to form a layer one molecule thick, without interaction between the drug molecules themselves.

The equation of Langmuir in its linear form is

$$C_{eq}/(x/m) = 1/k1 \cdot K_2 + C_{eq}/K_2 \qquad (1)$$

where C_{eq} is the concentration of drug found in the supernatant at equilibrium and x/m is the amount of drug in μg adsorbed per mg of nanoparticles. The constant K_2 is obtained from the slope of a plot of $C_{eq}/(x/m)$ against C_{eq} (Fig. 4) and determines the maximum amount of drug, in μg, that can be adsorbed per mg of nanoparticles. We found a maximum loading capacity of 30.8 μg HP/mg PIBCA nanoparticles when HP was added to preformed nanoparticles at pH 6.2; this maximum was reached for a concentration close to 1 mg HP/ml.

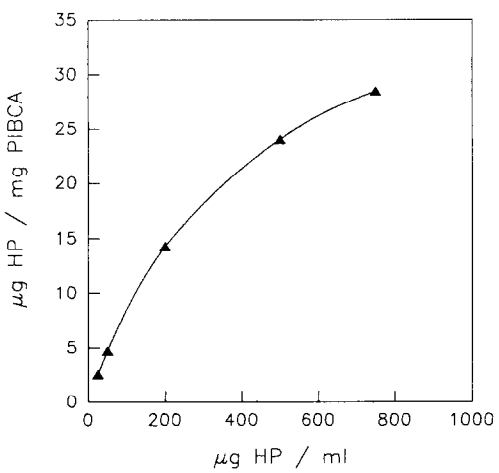

Fig. 3. Adsorption of hematoporphyrin (μg HP/mg PIBCA) onto PIBCA nanoparticles as a function of the HP concentration in the incubation medium (pH 6.2).

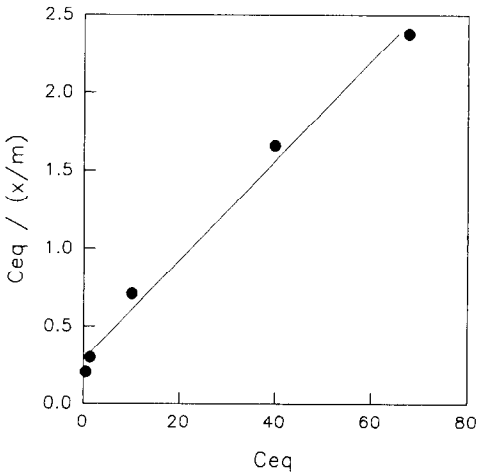

Fig. 4. Langmuir isotherm of the adsorption of hematoporphyrin onto PIBCA nanoparticles, at pH 6.2.

On the other hand, Illum and collaborators (1986) have observed that the adsorption technique used to prepare rose Bengal-loaded nanoparticles resulted in lower carrier capacity as compared to the incorporation process where the drug was added during the polymerisation step. Indeed, when the drug is associated with nanoparticles by an adsorption process, as in our case for hematoporphyrin, its fixation is likely to be restricted to the external surface of the polymeric support. However, owing to the high porosity of the particles (Kante et al., 1980), one cannot exclude a partial adsorption of the drug to polymeric sites more distant from the outer surface of the polymer.

Release of HP from nanoparticles

When nanoparticles were diluted 10 times in phosphate buffer at pH 5.9, 20% of the drug initially adsorbed onto nanoparticles was released during the first 20 min of incubation whereas

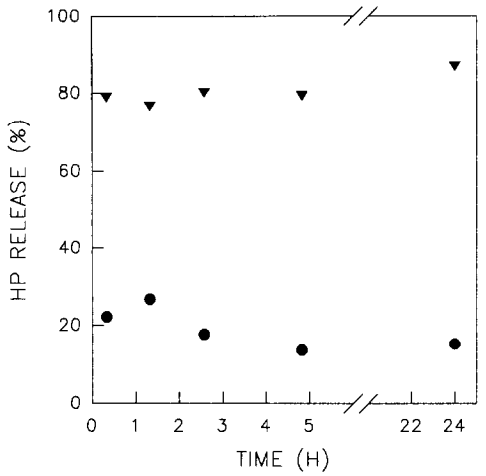

Fig. 5. Release of hematoporphyrin from PIBCA nanoparticles prepared at pH 5.9 and diluted 10 times in phosphate medium buffered at pH 5.9 (●) or at pH 7.4 (▼) (initial concentration of HP: 180 μg/ml, 13.5 μg HP adsorbed/mg PIBCA).

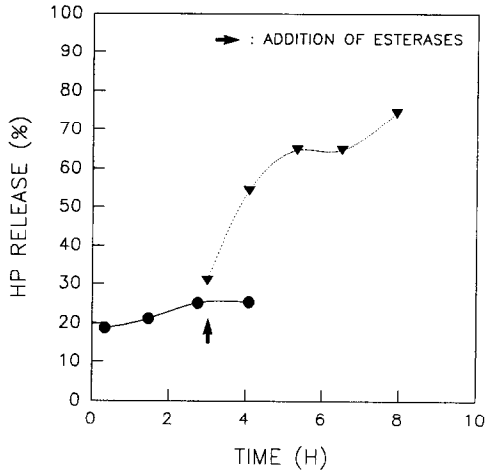

Fig. 6. Release of hematoporphyrin from PIBCA nanoparticles prepared at pH 5.9 and diluted 10 times in phosphate medium buffered at pH 5.9 (●). The incubation medium was enriched with esterases (100 μg/ml) after 3 h (▼) (initial concentration of HP: 170 μg/ml, 13.6 μg HP adsorbed/mg PIBCA).

when the same experiment was performed in phosphate buffer adjusted to pH 7.4, as much as 80% of the nanoparticles drug content was desorbed over the same time interval (Fig. 5). As mentioned above, this discrepancy may be attributed to the lower affinity of the anionic forms of HP for PIBCA nanoparticles at pH 7.4. This results in a shift of the equilibrium conditions between adsorbed and free drug when nanoparticles prepared at pH 5.9 are diluted in a medium at pH 7.4. Also, a 10-fold dilution of the nanoparticle suspension at the same pH (5.9) was sufficient to induce a slight disturbance of the equilibrium conditions leading to the release of 20% of the adsorbed drug.

Moreover, no significant drug release was observed after 20 min of incubation, indicating that

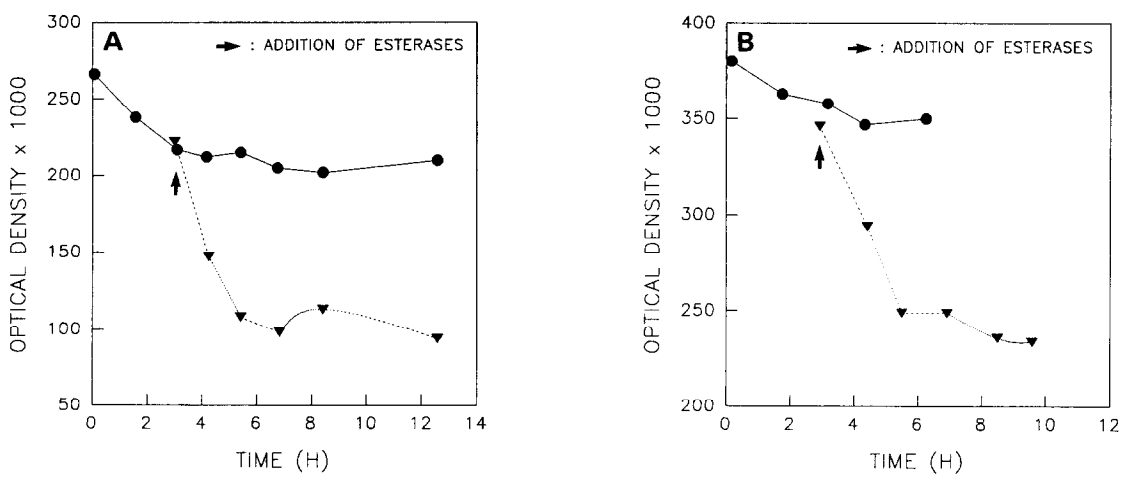

Fig. 7. Turbidity of PIBCA nanoparticles prepared at pH 5.9 and diluted 10 times in phosphate medium buffered at pH 5.9 (●). The incubation medium was enriched with esterases (100 μg/ml) after 3 h (▼); (A) unloaded nanoparticles, (B) nanoparticles loaded with hematoporphyrin.

there was no further diffusion of the drug from the nanoparticles to the external medium. In view of that observation, one can assume that drug interaction with nanoparticles proceeds mainly via an adsorption process at the surface of the nanoparticles. However, it is conceivable that a part of the drug was adsorbed to sites more deeply seated into the pores present at the surface of the nanoparticles.

The kinetics of release of hematoporphyrin under physiological conditions are close to the results obtained by Illum and collaborators (1986), who reported for rose Bengal adsorbed to nanoparticles a fast initial release and a high extent of release in phosphate buffer at pH 7.4.

When esterases (100 μg/ml) were added to the incubation medium at pH 5.9 after equilibrium conditions have been reached, the release of HP increased gradually from 20 to 75% of the initial drug content (Fig. 6). It is well established that esterases are responsible for the hydrolysis of the ester side chains of the polymer leading to the progressive solubilization of the polymeric chains (Lenaerts et al., 1984). In our case, this results in the bioerosion of the polymer from the external surface to the inner core inducing the release of HP adsorbed at the surface or into the superficial pores of nanoparticles.

Nanoparticle bioerosion was confirmed in our experiments by the decrease of nanoparticle turbidity as soon as esterases were added to the incubation medium (Fig. 7). It was observed that the absorbance of nanoparticles loaded with HP was higher as compared to unloaded nanoparticles, because of the slight absorption of HP at 470 nm, as reflected in Fig. 7.

Conclusion

PIBCA nanoparticles of hematoporphyrin were prepared by adsorption of the drug on the surface of the polymer rather than by the incorporation process, because of the insolubility of HP at the acidic pH values required for adequate polymerisation. This method of drug loading resulted in a poor carrier capacity because the drug was adsorbed mainly at the surface of the nanoparticles. Drug desorption resulted essentially from a shift in the equilibrium conditions between free and adsorbed drug, following dilution of the nanoparticle suspension, modification of the pH conditions or enzymatic hydrolysis of the polymer by esterases.

Due to the rapid release of HP from nanoparticles after dilution in buffer maintained at physiological temperature and pH, it appears that hematoporphyrin-loaded polyalkylcyanoacrylate nanoparticles would not be useful for in vivo applications. However, other porphyrin derivatives and second-generation photosensitizers showing solubility compatible with the pH needed for the polymerisation process are under investigation for incorporation into polyalkylcyanoacrylate nanoparticles, with the hope of allowing a more progressive release of the drug following degradation of nanoparticles by the enzymatic process.

Acknowledgments

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