Mechanism of Action and Surfactant Influence During Chemotherapy of Brain Tumour Using Doxorubicin-Loaded Poly(butyl Cyanoacrylate) Nanoparticles

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ABSTRACT

Doxorubicin (Dox) loaded in poly(butyl cyanoacrylate) nanoparticles (PBCA NP) coated with polysorbate 80 or poloxamer 188 produced a high antitumour effect against orthotopic 101/8 glioblastoma in rats. In the present study, the protein adsorption pattern of polysorbate 80- or poloxamer 188-coated DOX-loaded PBCA NP after incubation in rat plasma was investigated. It was shown that the protein adsorption patterns of both types of NP were similar, exhibiting a high adsorption of apolipoprotein ApoA-I (ApoA-I). It is, therefore, hypothesized that the efficacy of these formulations may be related to the interaction of ApoA-I on the particle surface with the scavenger receptor SR-BI expressed at the BBB. Accordingly, these results suggest a correlation between the adsorption of ApoA-I on the nanoparticle surface and the resulting delivery of drugs to the brain by these particles.

Keywords: apolipoprotein A-I, glioblastoma, nanoparticles, poly(butyl cyanoacrylate), surfactant

1 INTRODUCTION

Brain tumours are difficult to treat due to insufficient drug delivery across the BBB. The routine approaches applied for the enhancement of drug concentrations in the brain involve craniotomy and are highly invasive.

Recent findings suggest that non-invasive systemic drug delivery to the brain may be achieved by means of the nanoparticles. It has been shown that the effectiveness of brain delivery dramatically depends on the surfactant used for coating of the NP. Thus, coating of PBCA NP with polysorbate 80 (Tween® 80 = Ps80) enables enhanced brain concentrations of a number of drugs that are unable to cross the BBB in free form [1]. The effectiveness of this drug delivery system was most clearly demonstrated when doxorubicin bound to PBCA nanoparticles coated with polysorbate 80 produced a high anti-tumour effect against intracranial glioblastoma in rats [2]. It is known that the biodistribution of the intravenously administered colloidal carriers is governed by the pattern of proteins adsorbed on their surface from the blood. It was first hypothesized that drug delivery to the brain by means of PBCA NP coated with Ps80 is enabled by the ability of this surfactant to selectively promote adsorption of plasma apolipoproteins E and B on the surface of the NP. The interaction of these apolipoproteins with the LDL receptors at the BBB promotes receptor-mediated endocytosis of the drug-loaded NP by the endothelial cells forming the BBB [1].

Further studies, however, suggested that the mechanism of brain delivery by means of the NP may not be so unequivocal. It was shown that coating of PBCA NP with poloxamer 188 (Pluronic® F68 = F68), could also enhance the anti-tumour effect of Dox against intracranial glioblastoma [3]. Moreover, lipid drug conjugate (LDC) nanoparticles could also reach the brain; however, these particles did not adsorb apolipoproteins E and B, but apolipoproteins A-I and A-VI were found in considerable amounts [4]. Taken together, these facts indicate that the mechanisms by which particles deliver drug to the brain may be versatile depending on their surface properties. The objective of the present study was to further investigate the phenomenon of drug delivery to the brain using PBCA nanoparticles coated with different surfactants.

2 MATERIALS AND METHODS

2.1 Chemicals

n-Butyl-2-cyanoacrylate (Sicomet® 6000) was obtained from Sichel-Werke (Hanover, Germany). Doxorubicin hydrochloride (Dox) was from Sicor (Rho, Italy). Other chemicals were purchased from Sigma (Steinheim, Germany).

2.2 Preparation of doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles

Doxorubicin-loaded nanoparticles (DOX-PBCA) were prepared by anionic emulsion polymerisation: 200 µl of n-butyl-2-cyanoacrylate were added under constant stirring at 500 rpm to 20 ml of the polymerization medium consisting of 1 % solution of dextran 70,000 in 0.01 N HCl. After 30 minutes, Dox was added to a concentration of 0.25 %. Stirring was continued for 2.5 h, and then the

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polymerization was completed by neutralization with 0.1N NaOH. The nanoparticle suspension was filtered through a sintered glass filter and freeze-dried after addition of 3 % mannitol.

2.3 Characterization of nanoparticles

The particle size and zeta potential were measured using a Malvern Zetasizer 3000-HS_A (Malvern, Worcs, UK).

The percentage of the bound drug was calculated by the estimation of the free drug amount after its separation by ultrafiltration using centrifuge filter devices (Ultrafree MC, 100,000 NMWL, Millipore, USA). The concentration of the free drug in the filtrate was assessed by HPLC.

The polymerisation yield was detected by gas chromatography. For this the polymer (PBCA) in the nanoparticle samples was hydrolysed by addition of NaOH, and the amount of resulting n-butanol was assessed after extraction with dichloromethane.

2.4 Determination of plasma protein adsorption by 2-D PAGE

2-D PAGE was performed as follows. In the first dimension, the rat plasma proteins were separated according to their isoelectric point. In the second dimension, the separation was carried out according to the molecular weight of the proteins (SDS-PAGE). Freeze-dried NP were resuspended in a 1 % solution of Ps80 or F68 so that the concentration of Dox in the samples was 2.0 mg/ml and then incubated at room temperature for 30 min. After that 1 ml of the nanosuspension was incubated with 3 ml of citrate-stabilized rat plasma (5 min/37°C). Then the particles were separated from the medium by centrifugation and washed with 3 x 1 ml of bidistilled water. The gels were cast with a gradient from 9 % to 16 % polyacrylamide using 0.4 % N,N'-methylene-bis-acrylamide as a cross-linker. After SDS-PAGE, the gels were silver-stained and scanned using a laser densitometer. The protein adsorption patterns were analyzed using the MELANIE 3 software. The amount of proteins adsorbed was assessed in a semi-quantitative manner based on spot size and intensity of silver staining. The matching of rat plasma proteins was performed using the reference maps.

2.5 In-vivo studies

Orthotopic tumour model. Tumour implantation was performed as described [2]. Male Wistar rats were deeply anesthetized. Through a midline sagittal incision a burr hole of 1.5 mm in diameter was drilled in the scull at a point 2 mm posterior to the right coronal suture and 2 mm lateral to the sagittal midline. Tumour implants (approx. 10^6 cells) from frozen stock were introduced into the bottom of the right lateral ventricle. After development of pronounced clinical signs of the disease (usually at day 14), the animals were sacrificed by carbon dioxide asphyxiation. The brains were removed, the tumours were excised. Then fresh tumour implants (~10^6 cells) were inoculated into the brains of experimental animals, as described above.

Drug treatment. Tumour-bearing rats were randomly divided into groups (n = 10 - 23) and received one of the following formulations: 1) Dox solution in saline (Dox), 2) Dox solution in 1 % F68 (Dox+F68), 3) Dox solution in 1% Ps80 (Dox+Ps80), 4) Dox-PBCA coated with F68 (Dox-PBCA+F68), 5) Dox-PBCA coated with Ps80 (Dox-PBCA+Ps80). Untreated animals were used as control. These preparations were injected i.v. into the tail vein using the dose regimen of 3 x 1.5 mg/kg on days 2, 5, and 8 post tumour implantation.

Coating of the NP with the surfactant was performed immediately before administration in animals. For coating, the freeze-dried formulations were resuspended in 1 % of an appropriate surfactant, and the suspensions were incubated for 30 min.

The animals were followed up for survival. The long-term survivors were sacrificed 100 days post tumour implantation and subjected to necropsy.

3 RESULTS

In the present study, loading of Dox in PBCA NP was achieved by the emulsion polymerization of n-butyl-2-cyanoacrylate in the presence of Dox. This procedure yielded NP with an average size of 202 ± 10 nm and zeta potential of -13.0 ± 2.1 mV; loading of Dox reached 65 ± 11 %; yield of PBCA was ~68 %. Surfactant coating did not significantly influence zeta potential or particle size.

The brain tumour model and the treatment regimen for the present study were chosen based on the results of the previous experiments, where this experimental protocol was used for the treatment of 101/8 glioblastoma using nanoparticulate Dox formulations [2, 3].

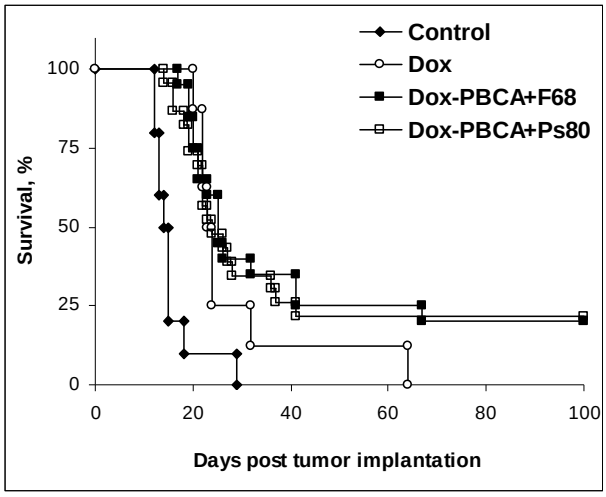

Fig. 1. Survival (Kaplan-Meier plot) of rats bearing intracranial 101/8 glioblastoma after i.v. administration of doxorubicin formulations

As shown in Fig. 1, all Dox formulations extended the survival times of the tumour-bearing animals when compared with control. However, long-term remission was achieved only in the groups treated with surfactant-coated NP. Dox-PBCA+Ps80 and Dox-PBCA+F68 enabled similar antitumour effects, producing long-term survival of 22 % (5/23) and 20% (4/20) animals, respectively. Necropsy of the long-term survivals performed 100 days post tumour implantation revealed no signs of tumour growth. Solutions of Dox in saline, 1% F68, or 1 % Ps80 produced only single long-term survivors.

The plasma protein adsorption patterns of DOX-loaded PBCA NP coated with F68 or Ps80 are shown in Fig. 2. It can be seen that the protein adsorption patterns of these formulations exhibited both qualitative and sometimes even quantitative concurrence in spite of the different structure of the surfactants used for coating. Thus, both types of the NP adsorbed high amounts of the apolipoproteins ApoA-I and ApoJ. Furthermore, the amounts of albumin and IgG were comparable. Differences could be seen in the adsorption of antitrypsin and transferrin (not shown). The protein patterns of the uncoated NP loaded with Dox differed considerably from the adsorption pattern of the surfactant-coated NP. The amount of albumin adsorbed on the surface of uncoated particles exceeded 50 % of the total amounts of adsorbed proteins (data not shown).

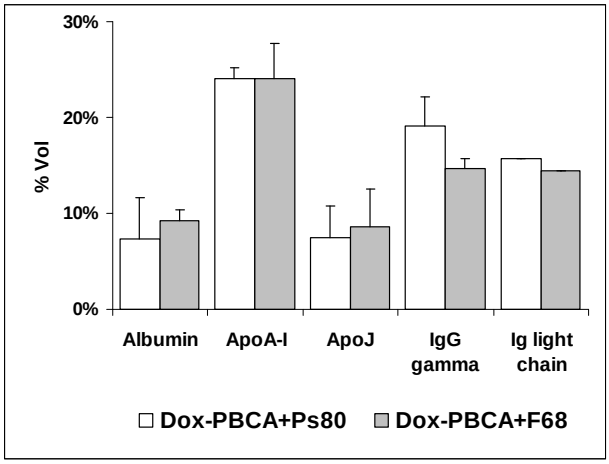

Fig. 2. Similarity of plasma proteins pattern adsorbed by DOX-loaded PBCA nanoparticles coated with polysorbate 80 (Dox-PBCA+Ps80) or poloxamer 188 (Dox-PBCA+F68) in rat plasma (mean ± sd, n = 4)

The plasma protein pattern of the empty NP coated with the same surfactants did not exhibit the same extent of similarity as the DOX-loaded NP. Thus, the presence of F68 led to the adsorption of antitrypsin, α-1-HS-glycoprotein, and transferrin. These proteins could not be detected on the Ps80-coated NP; instead, a considerable amount of fibrinogen (13.5 %) was found. Both surfactants remarkably enlarged the amounts of the apolipoproteins ApoA-I, ApoA-IV, and ApoC-III on the surface of the empty particles.

4 DISCUSSION

The mechanism(s) of the drug delivery to the brain by means of the NP is not fully elucidated. Doxorubicin bound to PBCA NP coated with Ps80 and F68 was highly effective against the 101/8 glioblastoma [2, 3], which is indicative of the ability of these carriers to deliver therapeutic amounts of the drug to the tumour site. As mentioned above, Ps80 was presumed to enable a receptor-mediated endocytosis of the NP at the BBB due to the interaction of the apolipoproteins E and B adsorbed on their surface with the LDL receptors at the BBB [1, 5, 6]. At the same time, the efficacy of F68 coating was unexpected. Indeed, in the previous study F68 coating failed to enhance delivery of dalargin loaded in PBCA NP across the BBB, whereas Ps80 with coating was very effective [6].

However, as seen from Fig. 1, the antitumour effects of Dox-PBCA+F68 and Dox-PBCA+Ps80 were found to be similar: both formulations considerably increased the median survival rates and produced ~20 % of long-term survivors [3]. This similarity correlated with the close resemblance of plasma protein adsorption patterns shown by the results of the 2-D PAGE study (Fig. 2). Furthermore, apolipoprotein E presumed to be a key factor for the interaction of the NP with the BBB could not be detected on the surface of either Dox-PBCA+Ps80 or Dox-PBCA+F68. Instead, ~24 % of ApoA-I was found. Interestingly, this result correlates with the previous observation of Gessner et al. [4] that lipid drug conjugate (LDC) NP, which could penetrate into a murine brain adsorbed high amount of ApoA-I and only a tiny amount of ApoE. These authors suggested that ApoA-I prevents hepatic uptake of the NP, and thus their brain uptake is enhanced. However, in the case of the Dox-loaded PBCA NP this mechanism is unlikely, since, as shown by the pharmacokinetic studies, Ps80 did not have a considerable influence on the hepatic uptake of this formulation [7, 8].

It is now hypothesized that drug delivery to the brain is mediated by the interaction of ApoA-I adsorbed on the particle surface with the so-called scavenger receptor class B type I (SR-BI) expressed on the membranes of the endothelial cells of brain capillaries [9]. This receptor mediates lipid uptake from ApoA-I to cells. The mechanism of the lipid uptake by ApoA-I involves efficient and selective receptor-mediated transfer of the lipids to cells thus being different from that of the classic receptor-mediated endocytosis.

The uptake of DOX into the cells may be assisted by circumvention of the transmembrane efflux pump P-glycoprotein. This phenomenon is enabled by simultaneous release of Dox and degradation product of PBCA (polycyanoacrylic acid), which form an ion-pair capable of crossing the cell membrane without being recognized by P-glycoprotein [10]. On the other hand, circumvention of the

P-gp may be assisted by retention of the NP due to their interaction with the SR-BI receptor at the cell walls.

It is noteworthy that the patterns of protein adsorption of the empty PBCA NP coated with either Ps80 or F68 were different. This observation suggests that the surface properties of the NP are influenced not only by the coating surfactants but also by the drug present on the surface. Furthermore, these facts also indicate that particles with different surface properties may deliver drug to the brain by different pathways. As mentioned above, apolipoproteins ApoA-I and ApoE/B interact with the BBB via different receptors. It appears, therefore, that both mechanisms can be employed for the nanoparticle-mediated transport of drugs into the brain. Thus, the results obtained in this study highlight the versatility of the mechanisms by which plasma proteins influence the biodistribution of the NP.

This hypothesis is supported by the very recent findings showing the involvement of ApoA-I in the interaction of different NP with the BBB [11-13].

5 CONCLUSIONS

The results of the present study suggest that the delivery of doxorubicin to the brain by means of PBCA nanoparticles coated with poloxamer 188 and polysorbate 80 is enabled by the enhanced adsorption of apolipoprotein A-I from the blood and its interaction with the scavenger receptor SR-BI located at the BBB, which promotes NP contact with the blood brain vessel endothelial cells.

The 2-D PAGE analysis of the plasma protein adsorption demonstrated that the pattern of adsorption is influenced by all constituents manifested on the nanoparticle surface.

Finally, this study indicates that mechanisms of drug delivery to the brain by means of the nanoparticles are versatile and depend on the surface properties of these particles.

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