Formulation of Benzothiazinones in Bovine Serum Albumin Nanoparticles

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Summary

The development of albumin-based nanoparticle drug delivery systems is an area that has garnered increasing interest over the years. While an injectable formulation (Abraxane®) has already achieved success, development in terms of pulmonary drug delivery has been limited due to lack of knowledge about how albumin behaves in the lungs. Previous studies on the clearance, biocompatibility and biodistribution of albumin nanoparticles have demonstrated their ability to target drug delivery to the lungs. The next step is to examine drug loading of albumin nanoparticle formulations. This study has investigated the interactions of albumin with two novel anti-tuberculosis compounds, IR 20 and IF 274, from a class of drugs called benzothiazinones. Solubilisation studies with these model hydrophobic compounds in albumin solutions showed that an increase in drug surface area promoted additional solubilisation, e.g. from ~40% to ~70% of 1 mg of IR20. Benzothiazinone (BTZ)-loaded albumin nanoparticles were successfully manufactured (size ~100 nm) from the solutions, illustrating how compounds that interact with albumin can be incorporated into a nanoparticulate albumin system for inhaled drug delivery.

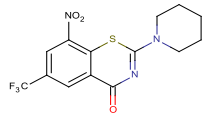
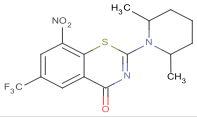
Introduction

Nanoparticles have shown potential for providing controlled drug delivery to the lung for the past few years [1]. Protein-based nanoparticulate systems, in particular, are of great interest as they have been shown to be non-toxic, non-immunogenic and reproducible and demonstrate ease of manufacture in terms of scale up [2]. Albumin is one such protein that has exhibited some of these properties and therefore makes it an attractive candidate for nanoparticle formulation. Albumin nanoparticles are already in use in clinical medicine in the Abraxane® formulation. Despite this, there are few studies exploring their potential as an inhaled formulation.

The biocompatibility, clearance, and biodistribution profiles of albumin have been investigated previously [3]; showing with *in vivo* cell uptake studies that albumin nanoparticles (sized ~180 nm) remained in the lungs longer compared to albumin in solution with 64% vs 40.6% respectively at 48 hours. This study also looked at levels of albumin nanoparticle associated radioactivity located within the lungs and found more activity within the lung tissue (23.3 ± 4.7%) compared to the lung fluid (16.1 ± 4.4%) at t = 48 hours. These studies have provided important information regarding the fate of albumin nanoparticles in the lungs and their potential for targeting drug delivery to the lung via inhalation. Therefore, they provide promise as a potential carrier for anti-tuberculosis drugs with alveolar macrophages being their therapeutic target. However what still remained unclear with these studies was whether therapeutic agents can be loaded into albumin nanoparticles.

The aim of this study therefore was to investigate albumin nanoparticles as a potential carrier for drugs to be delivered to the lungs. The model drugs used in this study is from a class of anti-tuberculosis drugs called benzothiazinones (BTZs) which are very hydrophobic (Table 1). The lipophilicity of the BTZs has been shown to be important for maintaining their activity, hence BTZs often have low aqueous solubility and poor oral bioavailability [4]. This has prompted investigation into the inhalation route of administration as an alternative to improve bioavailability. Solubilisation studies were carried out to assess the interaction between albumin and the BTZs. This was carried out by using UV spectroscopy to detect how much compound had been solubilised by albumin over time (t = 0, 1, 4 and 6 days). This was followed by the manufacture and characterisation of BTZ-loaded albumin nanoparticles using a modified approach to a reported desolvation method [3,5].

Table 1. Molecular weight, structure, and aqueous solubility of benzothiazinone compounds. Information was provided by Professor Peter Imming and Mr Adrian Richter, Martin-Luther-University Halle (Saale), Germany.

Compound	Molecular weight	Molecular formula	Structure	Aqueous solubility (mM)
IR 20	359.32	$C_{14}H_{12}F_3N_3O_3S$		0.373
IF 274	387.38	$C_{16}H_{16}F_3N_3O_3S$		0.087

Experimental Methods

Solubilisation studies

Stock solutions of bovine serum albumin (BSA) (fatty acid-free, Sigma—Aldrich, Germany) in water and Tris buffer (0.01M, pH 8.9) respectively (50 mg/mL) were prepared. Negative controls consisted of water and Tris buffer without BSA. BSA solutions and negative controls (2 mL) were added to separate vials containing 1 mg BTZ compound and incubated at 37°C for a total of six days. Samples of the supernatant were removed and UV absorbance readings were taken at 347 nm at time points t = 0, 1, 4 and 6 days. Previously constructed calibration curves were used to determine the amount of drug solubilised by each solution and calculated as a percentage of the original drug content.

Preparation of BTZ-loaded BSA nanoparticles

To produce BTZ-loaded albumin nanoparticles, a stock solution of 50 mg/mL BSA in 0.01M Tris HCl buffer (pH 8.9) was prepared. The BSA solution (2 mL) was incubated with BTZ compound (1 mg) in a microcentrifuge tube, with a blank also included as a control, followed by incubation at 37°C for 4 days to promote drug solubilisation. Following incubation, 0.5 mL was removed and placed in a clean vial. After the addition of 12.5 µL NaOH, 2.0 mL of ethanol was added drop wise to the stirred protein solution. Nanoparticles were cross-linked by addition of 23.6 µL 10% glutaraldehyde in water and overnight stirring. Particles were purified by at least 4 cycles of spin filtration (30kDa MWCO) into phosphate buffered saline (PBS).

Characterisation of BTZ-loaded BSA nanoparticles

Particle size and polydistribution index (PDI) values were measured by photon correlation spectroscopy (PCS) using the Zetasizer Nano Series ZS (Malvern Instruments, Malvern, UK). Zeta potential was measured in water and PBS and measured at a concentration of 20 µg/mL. Nanoparticle concentration was determined gravimetrically.

Results

The temporal profile of BTZ solubilisation by BSA was assessed by incubating ~1 mg compound in different solutions with and without BSA at 37°C over a six day period (Figure 1). The solubility profile in Tris and aqueous BSA solutions was assessed to determine whether drug solubilisation by albumin can occur in a buffer solution at pH 8.9, as used to generate albumin nanoparticles. Approximately 40% IR20 was solubilised by albumin over the six day period resulting in an approximate drug loading of 0.4% (w/w), while ~10% of IF 274 was solubilised.

Figure 1. Solubility profile of IR20 and IF 274 in three different media incubated at 37°C: Tris, Tris with 50 mg/mL BSA or distilled water with 50 mg/mL BSA (2 mL total volume). Results are expressed as a percentage of the original mass (~1 mg) at t=0. Results reported are the mean and standard deviation from n=3 experiments.

No significant differences were observed between solubilisation profiles in BSA/water and BSA/Tris solutions and so for further solubilisation studies BSA/Tris solutions were used only. No significant dissolution was observed in absence of BSA. For IR 20, significant differences were observed between solubilisation in BSA vs in Tris from time point t = 1 day onwards. For IF 274, significant differences were only observed from t = 4 days onwards.

To overcome the poor dissolution profile of IR 20 and IF 274, a modified experiment was designed, which involved dissolving the BTZ first in chloroform, adding the solution to a glass vial and evaporating the chloroform to form a thin film of drug prior to incubation with BSA. This modification was carried out to determine whether an increase in drug surface area would increase the rate and/or extent of IR 20 solubilisation. As hypothesised, the extent of solubilisation of IR 20 by BSA increased to ~70% and for IF 274 increased to ~40% (Figure 2). It also took 2-3 days to reach maximum solubilisation as opposed to the original method where it took up to six days.

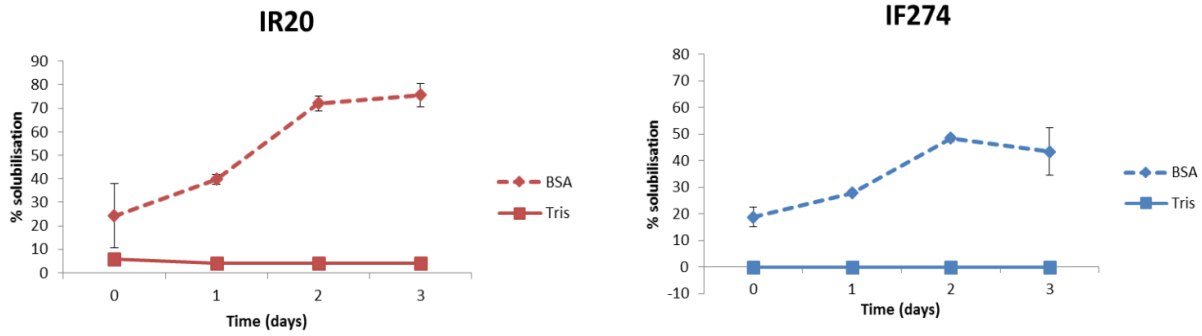


Figure 2. Solubilisation profiles of 1) IR 20 and 2) IF 274 using a film-forming approach with Tris only and Tris with 50 mg/mL BSA. Results are expressed as a percentage of the original mass (~0.5 mg) at t=0. Results reported are the mean and standard deviation from n=3 experiments.

BTZ-loaded BSA nanoparticles were produced using the desolvation method following drug incubation (1 mg) with a BSA/Tris solution (50 mg/mL; 2 mL) for four days to promote solubilisation. Nanoparticle sizes and zeta potential values of the drug-loaded nanoparticles were comparable to plain BSA nanoparticles, with low polydispersity (Table 2). No significant differences were observed for particle sizes between the BTZ-loaded and plain BSA nanoparticles. No significant differences were observed between particle sizes at t = 0 versus size measurements taken after one week. Visually, BTZ-loaded BSA nanoparticles looked similar to the control, remaining colloidally stable. Yield calculations were carried out following manufacture. This was done by calculating the BSA nanoparticle concentration (which was worked out gravimetrically) as a fraction of the theoretical particle concentration had there been a 100% yield i.e. had all of the BSA formed particles successfully. All particle batches were found to have a yield of 100%.

Table 2. Nanoparticle characterisation of BTZ-loaded albumin nanoparticles and control blank nanoparticles. Results reported are the mean and standard deviation from n = 3 batches.

Albumin nanoparticles	Zeta potential [mean ± SD (mV)]	Size @ 37^{o}C (nm) and [p.d.i]	Size after one week (nm) [p.d.i]
IR 20	-24.4 ± 3.1	99.9 ± 10.4 [0.12 ± 0.02]	106.0 ± 15.4 [0.1 ± 0.00]
IF274	-32.0 ± 3.5	100.0 ± 2.6 [0.11 ± 0.00]	104.3 ± 3.1 [0.09 ± 0.02]
Control	-23.8 ± 11.7	104.7 ± 2.9 [0.125 ± 0.016	104.4 ± 5.8 [0.12 ± 0.02]

Discussion

Albumin nanoparticles hold much promise as potential carriers for therapeutic agents for pulmonary diseases. The behaviour of albumin nanoparticles in the lungs has been studied previously [3], however more research is required in order to understand drug loading with albumin nanoparticles before its clinical application. Hence in this present study the incorporation of a model drug to albumin nanoparticles has been investigated.

IR 20 and IF 274 were found to reach a solubilisation of ~40% and ~10% respectively with BSA after six days. A drug loading of 0.4% (w/w) was achieved because a small amount of BTZ (1 mg) was incubated with 100 mg of albumin. This provides the basis of a viable formulation as the BTZs have activity in the nanomolar range [4] and as albumin is present in the body, metabolic processes exist to prevent accumulation [6,7]. These findings show that even fairly hydrophobic compounds like IR 20 have the potential to be loaded into an albumin nanoparticle-carrier system. To increase the drug loading further, a modification to the method of solubilisation was used to increase the degree of solubilisation to ~70%. By dissolving the drug in solvent and forming a film the surface area is increased, thereby increasing solubilisation velocity by BSA. The difference in solubility can be attributed to the increase in surface area available for reaction when the drug is introduced to the albumin solution. The extent of interaction of this hydrophobic compound with BSA provides promise for other compounds previously formulated for oral delivery with little success.

BTZ-loaded BSA nanoparticles were successfully manufactured with a size of ~100 nm. The size range desired for the albumin particles for this project is ~70-250 nm as it is within this size range that the retention and potential controlled-release properties of albumin upon drug loading can be explored [8]. Of course, particles of this size range are too small for direct delivery to the lungs and would require the design of a micron-sized carrier system [9]. By using a modified desolvation method however, incubation of the drug with BSA prior to manufacture is required and the findings from the solubilisation experiments show only a proportion of the drug will be loaded with BSA. An alternative method for incorporating the drug into BSA nanoparticles may need to be explored and further studies need to be carried out to further characterise the drug-loaded nanoparticles manufactured here. This includes investigating the dissolution profiles of the drug-loaded albumin nanoparticles. In order to understand more about the interaction between the BTZs and albumin, further drug-binding studies will be carried out looking at specific binding sites for which methods have been established [10].

Conclusion

This study has demonstrated that hydrophobic, BTZ compounds can be solubilised by albumin and loaded into albumin nanoparticles by using a modified approach to the desolvation method. These findings provide promise for developing drug-loaded albumin nanoparticles as a platform for the delivery of poorly soluble drugs to the lungs. Together with previous findings demonstrating their enhanced retention and targeting abilities, it is increasingly clear that albumin nanoparticles may be a promising platform for treating pulmonary diseases by the inhalation route.

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