Pharmaceutical nanocarriers for the formulation of poorly soluble drug

PhD thesis

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Introduction

Poor solubility of drugs has always been an issue for formulation scientists thus the search for novel excipients or techniques is an ever-important field in the pharmaceutical developments. Besides the conventional solubilizers or pH adjusters and methods in the past decades particle size reduction is one of the new techniques to enhance the solubility of poorly water soluble drugs.

Until now several colloidal carrier systems have been evaluated. One of the colloidal carrier systems is the human serum albumin based nanoparticles. Human serum albumin (HSA) has acclaimed a wide acceptance in drug formulation as a nanocarrier system of active pharmaceuticals ingredient (API). Contrarily to some conventional excipients it is biocompatible and well tolerated by the human organism without serious side-effects, such as toxicity. The binding of drugs to serum proteins is particularly important because it affects both the activity of drugs and their disposition. It has been shown that the *in vitro* binding of poorly soluble drugs to HSA during the formulation can significantly increase the solubility of the drug allowing the API to be dissolved in therapeutically effective doses in an adequate aqueous dosage form to be used for parenteral administration. Further research in the field proved that the use of preparations containing albumin nanoparticles does not only improve the solubility of the drug, but can also exhibit other beneficial effects, such as targeted therapy.

Another type of colloidal carrier systems is the solid lipid nanoparticles (SLN). In the past few years, the mentioned lipid-based nanoparticles (LNP) can improve both the water solubility and the bioavailability of drugs. Out of the investigated systems, solid lipid nanoparticles (SLN) offer unique properties, such as the few hundred nanometers particle size and high drug loading capacity due to their large surface area. Such SLN formulations have proved their viability in dosage forms intended for various routes of administration (oral, parenteral, dermal, ocular) and have led to the solution of various solubility-related problems of poorly soluble drugs. Furthermore, SLN can improve the effectiveness of several drugs (ketoconazole, baicalin, tobramycin) because it can provide protection for the drug against the degradation and therefore prolong exposure of the drug by controlled release.

Objectives

Poor water solubility of APIs is a major issue in pharmaceutical development. This issue is prominently important in formulating liquid dosage forms and is underlined by the fact that almost 30% of the marketed APIs have solubility problems.

Voriconazole, the model active pharmaceutical ingredient, exhibits low water solubility, making the drug difficult to administer in liquid preparation, so nanoparticles can be good candidates to encapsulate VCZ, potentially increase its water solubility and its therapeutic efficacy in the treatment of fungal infection.

1) The aim of the study was to prepare and investigate the critical parameters of producing voriconazole-loaded nanoparticles (VCZ-NPs). In order to achieve the set goals, the aims was to study the effect of the applied organic solvent, concentration of VCZ, the concentration of the HSA solution and the ratio of the organic and aqueous solutions on the prepared nanoparticles. In additional, my aim was to investigate and optimize critical process parameters, such as homogenizing pressure, homogenizing cycle number and the method of organic solvent removal. Finally, my aim was to apply the optimized technology and characterize VCZ-NP by particle size, particle size distribution, loading capacity and in vitro dissolution.

2) The other objective of the study was to formulate and evaluate solidlipid nanoparticles of VCZ (VCZ-SLN) and to determine the critical parameters of preparation. In order to achieve these aims the effect of the applied solid lipid, the concentration of active pharmaceutical ingredient (API) and the concentration of lipid on the produced nanoparticles was investigated. Furthermore, my aim was to optimize the high pressure homogenization method to prepare VCZ-SLN, to apply the optimized technology in the preparation of the VCZ-SLN and characterize the VCZ-SLN by particle size, particle size distribution, loading capacity and *in vitro* dissolution.

Methods

Preparation of VCZ loaded albumin nanoparticles, nab™ technology

VCZ-NPs were prepared by nab™ technology. During the determination of the homogenization parameters various organic solvents (isooctane, chloroform) and various ratios of organic solvent and aqueous solution of HSA were applied. First 10, 20 or 50 mg of voriconazole was dissolved in 1 ml organic solvent. Human serum albumin infusion was diluted with Milli-Q water to 2 or 3%. Following the mixing of HSA solution with voriconazole solution, the mixture was prehomogenized for 3 min at 80 rpm using a Homorex mixer (Brogli& Co. AG.,Switzerland). The prehomogenized mixture was then further homogenized to form VCZ-NPs using an Avestin Emulsiflex B15 (Avestin,Germany). The resulting colloidal system was lyophilized (ScanVac Coolsafe TM) to remove the organic solvent residual.

Particle size of the nanoparticles is the most important character of the nanoparticles. The particle size was determined by Malvern Zetasizer Nano Zs (Malvern Istruments Ltd, UK).

Drug incorporation efficiency was determined after the lyophilized nanoparticles were reconstituted in 2.5 ml of water and filtered through 0.2 μm microporous syringe filters. HSA was denaturated with 2.5 ml of acetonitrile. The concentration of voriconazole was measured by HPLC (Agilent 1100) equipped with an Agilent C18 column (4.6 mm × 150 mm, 5 μm). The injection volume was 10 μl, the mobile phase of 70% acetonitrile and 30% 0.05 M sodium dihydrogen phosphate buffer was run isocratically at room temperature at a flow rate of 0.7 ml/min, and voriconazole was detected by UV detector at 254 nm.

Differential scanning calorimetry (DSC) measurement was use to investigate the interactions between the VCZ and albumin.

In vitro dialysis test was applied to predict the *in vivo* behaviour of the VCZ loaded albumin nanoparticles.

Preparation of VCZ loaded lipid nanoparticles (VCZ-SLN)

VCZ-SLN were prepared by high pressure homogenization. Firstly, PPC (0.03 g) was dissolved in 0.5 g of ethanol and 0.0075 g of polysorbate 80 solution. In the preparation methods utilized Witepsol®W35, stearic acid and Compritol®888 ATO were selected as lipids. The weight of the applied lipid (0.25, 0.50, 0.75 g) was molten at 10 °C above their melting points and voriconazole was dissolved there to obtain a clear lipid phase. Later surface active agents were added to the molten lipid and to this mixture 10 ml of hot MilliQ water (70◦C) was added under constant stirring (magnetic stirrer). The mixture was prehomogenized for 3 min by Ultra-Turrax homogenizer (IKA®Works Inc., Germany) at 20,000 rpm. The prehomogenized mixture was then further homogenized to form VCZ-SLN using an Avestin Emulsiflex B15 instrument (Avestin Europe GmbH, Germany) utilizing 600 bar (26,106 psi) inlet pressure for 3, 4 or 5 cycles.

The mean particle size of the undiluted SLN dispersions and the PDI were obtained by photocorrelation spectroscopy.

Differential scanning calorimetry and Fourier transform infrared spectroscopy (FTIR, Alpha Bruker Corp., USA,) were applied to evaluate the possible incompatibility between the VCZ and utilized auxiliary materials.

Entrapped drug (IE) concentration was determined indirectly. Drug IE was evaluated by an ultrafiltration technique with a 100 kDa centrifugal filter device composed of a regenerated cellulose membrane. An aliquot (0.5 ml) of undiluted SLN dispersion was placed into an Amicon ultracentrifugal filter. The sample was centrifuged or 10 min at 12000g. The filtered aqueous phase contained the free API. The concentration of non-embedded drug was analysed by above described HPLC-UV method.

In vitro release of voriconazole from VCZ-SLN was investigated using a dialysis tube. Voriconazole concentrations of the sample were measured by the HPLC-UV method described above.

The antifungal activity was investigated by paper disc diffusion method. The antifungal activities of VCZ-SLNs were evaluated against C. glabrata and A. flavus.

Results

Results of VCZ loaded albumin nanoparticles

Primarily the influence of the critical parameters on nanoparticle formulation was investigated. In order to develop nanoparticles loaded with voriconazole, the parameters of the homogenization process were elaborated. Results show that the following experiment variables have an impact on the size and size distribution of the prepared nanoparticles: type of organic solvent, homogenization pressure, number of homogenization cycles, concentration of API, ratio of the organic and aqueous phase. Optimization studies aimed at determining the ideal number of homogenization cycles proved that 6 cycles are necessary to stabilize the polydispersity index and that further homogenization cycles had no effect on the studies parameter.

Particles smaller than ideal (50-150 nm) were shown to lack systemic effect due to the immediate diffusion to the cell after the parenteral administration. Therefore the optimized albumin nanoparticles particles size should be in the ideal range. Out of the studied parameters the applied pressure has the highest influence on the size of the formed nanoparticles during high pressure homogenization. A twofold increase in the concentration (10 mg–20 mg) of the initial voriconazole solution has a negligible effect on the diameter of the formed particles, while a twofold increase in the homogenizing pressure (900 bar, 1800 bar) results in a decrease of 8, 12 and 38% for the 10 mg, 15 mg and 20 mg samples, respectively. In conclusion to the optimization study a method for producing voriconazole loaded nanoparticles was elaborated. Optimized parameters are chloroform as organic solvent, 1800 bar homogenization pressure and at least homogenizing 6 cycles.

Following the determination of the optimal homogenizing parameters and organic solvent, the ideal ratio of albumin solution and voriconazole solution concentration had to be worked out. Particle size analysis and examination of the entrapment efficiency were utilizied to aid the development process.

The relationship between the drug concentration and the incorporated concentration of VCZ shows that fivefold increase of drug concentration in the initial API solution was resulted less than twofold increase of the entrapped drug concentration and the relative solubility of voriconazole in the nanoparticles. Decreasing the organic and water phase ratio resulted in smaller particle size, while increasing the concentration of human serum albumin from 2% to 3% increased the size. Higher HAS concentration induced foaming in the samples during the prehomogenization and high pressure homogenization leading to unpredictable and

unstable API concentration. Thus, it was concluded that the ideal concentration of HSA in water is 2%. Polydispersity index value can accepted in the range of 0.2–0.7, but it should be as small as possible. The smallest polydispersity index value and optimal particle size were achieved using 10 mg/ml VCZ concentration in chloroform and 2% HSA concentration with 1:15 phase ratio.

DSC analysis was performed to study the physical state of powders. DSC curve of VCZNPs showed only the albumin thermal characteristics and no peak of voriconazole. A possible explanation is that voriconazole did not exist in the nanoparticles in a crystalline structure rather bound to HSA. This is underlined by the curve of the physical mixture of VCZ and HSA where features of both are seen. The calorimetry study confirmed that produced nanoparticles are not a physical mixture and based on the results it can be assumed that voriconazole binds to the HSA during the process of the HPH.

Dynamic dialysis verified that the binding of voriconazole is reversible. In the course of the test, it was seen that half of the amount of the totally encapsulated VCZ was liberated in less than an hour.

Results of the VCZ loaded lipid nanoparticles

The primary set of experiments was aimed at investigating the influence of the key parameters such as HPH process on nanoparticle formation. Test results were consistent with those in the literature as 3–5 homogenization cycles with HPH at 300–1500 bar were needed to form nanoparticles. Most of the articles of previous authors used homogenization pressure of 600 bar to produce SLN. Therefore 600 bar homogenization pressure was applied during the development. It is commonly accepted, that increasing the number of homogenization cycles reduces polydispersity. Results shows that the PDI decreased with 36.5% if applied 5 cycles instead of 3 cycles, therefore 5 homogenization cycles were preferable to apply during the homogenization process.

The parameters of homogenization (pressure, number of cycles) not only influenced the average particle size of the nanoparticles but also the type of solid lipid. Results indicated that the type of solid lipid also had an impact on the size and size distribution of the prepared nanoparticles. At first, blank SLNs were investigated during the development. Stearic acid and Compritol® 888 ATO based delivery systems were not suitable carrier for VCZ. These lipids were not fit with HPH method. Since the average particle size of the samples prepared with stearic acid or Compritol®888 ATO size was more than 0.5 nm even after 5 homogenization cycles and demonstrated high polydispersity value (more than 0.8).

At each concentration of Witepsol®W35 the particle size was less than 300 nm. Smaller particle size can provide low irritation, and can increase the compatibility with ocular tissue therefore the selected solid lipid was Witepsol®W35. In summary, 600 bar homogenization pressure and at least 5 homogenizing cycles produced adequate SLN from the selected lipid, Witepsol®W35.

After the determination of the parameters of HPH the effects of the concentration of lipid and VCZ on particle size were investigated. Increasing the concentrations of VCZ increased the polydispersity. Therefore, it is better to apply a lower concentration of VCZ to avoid the broad size distribution. Increasing the lipid content resulted in larger particle size and higher value of PDI. Furthermore, higher lipid concentration presence of the same VCZ concentration produced lower encapsulation efficiency. Therefore lower lipid content was preferable to apply during the formulation of VCZ loaded SLN. The encapsulation efficiency was mostly dependent on the concentration of VCZ. While the concentration of VCZ increased twofold from 50 mg to 100 mg, the entrapment efficiency increased by 10%. These results suggest that 50 mg VCZ is ideal to form SLNs due to its low polydispersity and high encapsulation efficiency.

The developed solid lipid nanoparticles consist of 0.25 g Witepsol® W35 and 50 mg voriconazole. The particle size of the ideal voriconazole loaded nanoparticles were 182 ± 4.1 nm. The voriconazole concentration was 3.94 mg/ml therefore the lipid naoparticle formulation increased the solubility of voriconazole more than fortyfold.

DSC thermograms of VCZ-SLN were similar to empty nanoparticles, no peaks of VCZ, only the thermal characteristics of Witepsol®W35 were observed. A possible explanation is that VCZ did not exist in the nanoparticles in a crystalline structure rather encapsulated to nanoparticles.

In the Fourier transform infrared spectrum of the VCZ-SLN were no appearances or disappearances of any characteristic peaks. The spectroscopic changes showed that C-O-H bands and aromatic rings of VCZ interacted with the vehicles and this suggested that the chemical structure of VCZ had not changed after forming SLN.

Dynamic dialysis curves were presented that the encapsulated VCZ could be released from the SLN.

Antifungal study of VCZ-SLN was proved by disc diffusion test. Clear zone of inhibition was not observed in case of empty SLN. This suggested that the excipient has no antifungal activity against A. flavus and C. glabra. Clear zone of inhibition against A. flavus and C. glabra indicated antimicrobial efficacy of VCZ-SLN formulations.

Conclusions

My PhD study showed formulation development of voriconazole loaded nanoparticles. Two types of nanoparticles (albumin and lipid based) were used to solve a solubility problems of voriconazole.

Voriconazole was successfully bound to the human serum albumin by nab® technology and determined the key parameters of the described technology. Six homogenization cycles at 1800 bar were produced VCZ loaded albumin nanoparticles with 81.2±1 nm average particle size. A specific HPLC-UV method was developed to determine both the entrapped and a released API concentration. DSC analysis verified that produced nanoparticles are not a physical mixture and based on the results it can be assumed that voriconazole binds to the HSA during the process of the HPH. The applied dialysis test proved that the bound voriconazole is released from the nanoparticle. Although, the encapsulated concentration of VCZ was 69.7±4.2% and the water solubility was over 2 times greater than the API itself, it was far from the therapeutic concentration. Therefore, need to apply other nanoparticles to enhance the solubility of VCZ.

The other objective of the study was to formulate VCZ loaded lipid-based nanoparticles by HPH. Applying five homogenizing cycles at 600 bar pressure resulted in VCZ-loaded nanoparticles with low PDI from the selected lipid base (Witepsol® W35). The optimized SLN formulation encapsulated the 80 % of initial VCZ concentration and provided 40 folds increasing in water solubility of the drug. Both DSC analysis and FTIR spectroscopy results indicated the formation of SLN. Dialysis test and antifungal study verified that the embedded VCZ is released from nanoparticles. Despite of the fact the encapsulated voriconazole concentration (3.94 mg/ml) didn't reach the systemic therapeutic level (10 mg/ml), the released API could inhibit the reproduction of fungus. Therefore, the novel SLN drug delivery system represents a promising alternative treatment for local fungal infections.

Publications

Publication of PhD thesis

1. Füredi Petra, Kovács Kristóf, Ludanyi Krisztina, Antal István, Klebovich Imre (2016)
Development and characterization of voriconazole loaded nanoparticles for parenteral delivery
Int.J. Pharm. (510) 159-163.
(IF.: 3.994 (2015))

2. Füredi Petra, Pápay Zsófia Edit, Kovács Kristóf, Dalmadi Kiss Borbála, Ludányi Krisztina, Antal István, Klebovich Imre (2017)
Development and characterization of the voriconazole loaded lipid-based nanoparticles
J. Pharm. Biomed. Anal. (132) 184-189.
(IF.: 3.169 (2015))

Other publictions

3. Kovacs Kristóf, Jayannaa Prashanth K., Dukea Anna, Winnera Brittany, Negritoa Melaeni, Angalakurthia Siva, Yub Jorn C.C., Füredi Petra, Ludányi Krisztina, Sipos Péter, Rockwoodc Gary A. , Petrikovics Ilona (2016)
A Lipid Base Formulation for Intramuscular Administration of a Novel Sulfur Donor for Cyanide Antagonism
Curr. Drug Deliv. 13: pp. 1351-1357

(IF.: 1.446 (2015))

4. Pápai Katalin, Füredi Petra, Budai Marianna, Mike Zsolt, Ludányi Krisztina, Antal István, Klebovich Imre (2009)
Diétás étkezés hatása a ciprofloxacin biohasznosulására – in vitro vizsgálat
Gyógyszerészet (53) S85-S86.

5. Füredi Petra, Papai Katalin, Budai Marianna, Ludanyi Krisztina, Antal István, Klebovich Imre (2009)
Fluorokinolonok in vivo étel-interakciós vizsgálatai
Acta Pharm. Hung. (79) 81-87.