European Journal of Pharmaceutical Sciences 87 (2016) 112–117

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Development of a nanosuspension for iv administration: From miniscale screening to a freeze dried formulation

Kerstin J. Frank *, Georg Boeck

Pharmaceutical Development, Boehringer-Ingelheim Pharma GmbH & Co. KG, Birkendorfer Straße 65, 88397 Biberach, Germany

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 6 February 2016
Accepted 5 March 2016
Available online 9 March 2016

Keywords:
Nanosuspension
Intravenous
iv administration
Nanoparticle

ABSTRACT

The aim was to develop a nanosuspension of the poorly soluble BI XX. The nanosuspension is intended for intravenous (iv) administration in preclinical studies and should not cause any unwanted side effects. Thus, only stabilizers that are accepted for iv application should be used and isotonicity, euhydria and the absence of living microorganisms were targeted. Suspensions were prepared in a ball-mill (mixing mill MM 400 from Retsch). There were various vials used as containers; HPLC-vials were used for the small scale screening of stabilizers and injection vials for preparation of larger quantities of the nanosuspensions. Particle size distribution was analyzed by laser diffraction measurement (Mastersizer 2000). Lyophilization was used for processing of the suspensions (Christ freeze dryer). Stable nanosuspensions (d90 remained <1 μm up to 7 days) were prepared with several FDA-accepted stabilizers. Freeze drying was evaluated for one formulation containing 2% of the API, 0.5% of arginine and 4% of mannitol. The particle size distribution before freeze drying and after redispersion was comparable. After milling for 2 h, no living microorganisms were detected in the nanosuspension. Various FDA accepted excipients were identified which resulted in stable nanosuspensions of BI XX. The most stable formulation was successfully freeze dried. It was proven that milling in the ball-mill decreases the presence of living microorganisms.

1. Introduction

In recent years, drug discovery research has been generating substance libraries of increasingly lipophilic and poorly water soluble molecules. This is caused by implementation of modern research methods like computer-aided drug design, high throughput screening or combinatorial chemistry. The poor solubility of those APIs is a major challenge across all stages of development. It is not only challenging to develop a suitable formulation for clinical phases which reveals a sufficient bioavailability. Even in the preclinical phase, poorly soluble APIs are problematic. For early PK-studies it is crucial to administer APIs intravenously for assessment of their pharmacokinetic characteristics (Chin et al., 2014). In these cases, poorly soluble APIs are typically delivered as solutions that contain high amounts of co-solvents or surfactants. However, as described by Rabinow et al. and Xiong et al., these described formulations might not only cause irritation at the site of injection, but can also lead to systemic shock reactions (Xiong et al., 2008; Rabinow et al., 2007). Another point to consider is the possibility of crushing out of the API due to dilution effects of the solubilizing excipients. This leads to an increased risk of embolism (Wong et al., 2008).

The administration of an aqueous nanosuspension with a d90 value below 1 μm is a promising alternative to avoid those unwanted side effects as the required amount of surfactants is decreased and no co-solvents or extreme pH-values are needed (Chin et al., 2014). In addition, a higher amount of API can be administered in a small volume since the solubility of the API is no limiting factor anymore. Due to the fact that the major part of the API is present as solid particles, a nanosuspension will be less prone to chemical degradation (e.g. caused by oxidative stress) than a solution (Moschwitzer et al., 2004). The chemical and microbiological stability can be even further improved by processing the nanosuspension further to a solid formulation (Van Eerdenbrugh et al., 2008).

Despite these benefits, some points need to be considered when developing a nanosuspension which is intended for iv administration as pointed out by Wong et al. There are only a small number of stabilizers accepted for parenteral use (Wong et al., 2008). Furthermore, most of the commonly used non-degradable polymeric stabilizers (e.g. HPC) are not accepted by the FDA (2015). Even though some anionic and non-ionic stabilizers are approved, they may still cause irritation in preclinical species, like described for poloxamer 188 (Wong et al., 2008). Thus their amount in the formulation should be kept to a minimum level.

For adjusting the tonicity of a nanosuspension, non-ionic excipients should be used, because salts may destabilize the nanosuspension by changing the electric charge of the particles. Mannitol has been

* Corresponding author at: Pharmaceutical Development, Boehringer Ingelheim Pharma GmbH & Co. KG, Germany.
E-mail address: Kerstin_Julia.Frank@Boehringer-Ingelheim.com (K.J. Frank).

http://dx.doi.org/10.1016/j.ejps.2016.03.003
0928-0987/

successfully applied without affecting the particle size distribution. Additionally it has been shown that it even acts as matrix forming excipient during further processing of the nanosuspension through spray-drying or freeze drying (Jacobs et al., 2000; Sigfridsson et al., 2007). Another prerequisite for parenteral applications is sterility. This poses a major challenge as sterile filtration, a pre-requisite for aseptic manufacturing, cannot be applied for nanosuspensions and hot steam sterilization or gamma radiation might induce particle growth (Nielloud and Marti-Mestres, 2000). Hence, in most publications on iv administration of nanosuspensions in preclinical studies, the suspensions are administered as non-sterilized formulations (Sigfridsson et al., 2007; Peters et al., 2000; Gao et al., 2010).

However, to minimalize the risk associated with the application of a nanosuspension, the aim of our study is to develop an isotonic, euhydric formulation that contains no particles in the micrometer scale and no living microorganisms. BI XX, a poorly soluble development compound, was used as model compound and a nanosuspension for iv application in a preclinical PK study should be developed.

This development included a literature search for suitable stabilizers that are accepted for iv application as well as a small scale screening of various formulations and evaluation of the bioburden of the formulation. In addition, the nanosuspension is processed to a solid formulation by freeze drying. Amphiphilic amino acids are investigated as new class of stabilizers for nanosuspensions. They are well accepted for iv administration and their successful application as stabilizers for proteins (inhibition of aggregation) by prohibition of hydrophobic interactions (Bolli et al., 2010) raises hope that they could be suited as stabilizers for nanosuspensions of hydrophobic APIs.

2. Material and methods

2.1. Material

A Boehringer Ingelheim internal development compound BI XX, which is a weak acid, was used as model compound for these studies. The physicochemical properties of are shown in Table 1. For preparation of the dispersion medium, deionized water was used. Polysorbate 80 (Dr. W. Kolb AG, Hedingen, Switzerland), polyethylene glycol 400 (NextPharma, Surrey, UK), mannitol (Roquette Freres, Lestrem, FR) arginine (Kyowa Hakko Bio CO. Ltd., JP), proline and benzalkonium chloride (both Sigma-Aldrich Taufkirchen, FRG) were used as excipients. The concentration and function of the excipients in the formulations are given in Table 2. Nanosuspensions were prepared in 2 ml HPLC-vials or 4 ml injection vials. For wet-milling, yttrium stabilized zirconium oxide milling pearls with a diameter between 0.3–0.4 mm from Getzmann (VMA-Getzmann GmBH, Reichshof, FRG) were used. For microbiological assessment, two incubation media (Casein-soja–peptone-bouillon (CaSo) and Thioglycolate-bouillon) were used. Both media were prepared in house with material from Sigma-Aldrich (Taufkirchen, FRG). Reference microorganisms were clostridium sporogenes, pseudomonas aeruginosa, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus brasiliensis*. These six microorganisms and the incubation media were chosen based on monograph "2.6.1 Sterility" of the European Pharmacopeia.

Table 1
Physicochemical properties of BIXX.

Property	Value
pK value (acid)	4.4
Logp	6.4
Solubility at pH 1 (mg/ml)	<0.001
Solubility at pH 7.4 (mg/ml)	<0.001
Solubility at pH 10 (mg/ml)	0.4
Solubility FaSSIF (mg/ml)	0.006
Solubility FeSSIF (mg/ml)	0.06
Solubility Ethanol (mg/ml)	>10
Melting point (°C)	174
Molecular weight (g/mol)	535
Morphology of crystals	Needles
Total polar surface area (Å)	85.2

Table 2
Overview about excipients.

Excipient	Concentration in nanosuspension (%)	Function
Benzalkonium chloride (BAC)	0.01	Conserving agent/stabilizer
Polysorbate 80	0.5–2.0	Stabilizer
Poyethylene glycol 400 (PEG 400)	0.5–2.0	Stabilizer
Mannitol	4	Matrix former, adjustment of tonicity
Proline	0.25–0.5	Stabilizer
Arginine	0.25–0.5	Stabilizer

2.2. Methods

2.2.1. Analysis of nanosuspensions

Particle size distribution (PSD) was determined through laser diffraction measurement. The Mastersizer 2000 device was used in combination with the Hydro 2000 μP dispenser (both Malvern Instruments, Worcestershire. UK) Deionized water was the dispersion medium in the measuring cell. The pH value of the nanosuspensions was measured with the pH-meter "SevenEasy" from Mettler Toledo (Giessen, FRG).

2.2.2. Miniscale screening

First, approximately 13 mg (or 28 mg) of the API was weighted into the 2 ml HPLC-vials. An aliquot of the dispersion medium (500 μl) was added and the vials were gently shaken to wet and pre-suspend the API. Afterwards, milling pearls (3.3 g) and the remaining medium were added (total medium volume: 1.3 ml for 2 ml). Vials were sealed by crimping. For milling, the vials were put in self-made holding devices (Fig. 1) which were mounted between the brackets of the Retsch ball mill (MM 400, Retsch GmbH, Haan, FRG). This device enables milling of 18 vials at once. Milling was performed for 2 h at 30 bps. Those settings were chosen based on a preliminary study in which particle size distribution at different milling times was evaluated (Supplementary data).

2.2.3. Manufacturing of nanosuspensions for lyophilization

For the preparation of larger volumes of nanosuspensions, 40 mg of API dispersed in 2 ml medium were milled in 4 ml injection vials for 4 h at 30 bps. Due to the larger size of the vials, milling was performed in different holding devices.

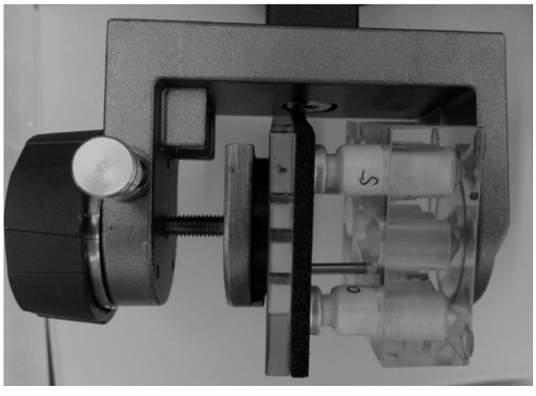

Fig. 1. Holding device for HPLC-vials intended for small scale screening. One device holds 9 vials and is mounted in the mill Figure 1 should be part of the main article.