Novel nanostructured drug delivery technology to enhance bioavailability, increase solubility and drug loading

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ABSTRACT

Nanotechnology provides new innovative solutions for original as well as generic applications in a wide variety of industries including pharmaceuticals, cosmetics, foods, nutraceuticals and home care. Nanoformulation is the reduction of particles size down to below 200 nm. This size reduction below 200 nm by using continuous flow nano precipitation technology led to 100s fold higher solubility and 10^{3-4} fold increased drug concentration in a stable colloid solution. In the paper we will discuss the perspectives and pharmacokinetic benefits of the nanostructured active molecules over the parent active molecules. The bioavailability of reference and nanostructured drug was determined after oral administration (30 mg/kg) in fasted state at Sprague-Dawley rats. Nanosized drug had an $AUC_{15\text{-}360min}$ value of 6412 µg·min/ml while this value after reference treatment was 940.1 µg·min/ml. The ratio of the two AUC values ($AUC_{15\text{-}360\ min\ (nanosized)}$ / $AUC_{15\text{-}360\ min\ (reference)}$) was 6.82.

Keywords: nanostructured drug delivery systems, increased solubility, enhanced bioavailability, continuous flow nano precipitation

1 INTRODUCTION

Over the last ten years, the number of poorly soluble drugs has steadily increased. Estimates state that 40% of the drugs in the pipelines have solubility problems. Progress in high throughput screening methods leads to an even greater amount of newly discovered drugs that have poor water solubility. Literature states that about 60% of all drugs coming directly from synthesis are nowadays poorly soluble[1,2]. Poor solubility in water correlates with poor bioavailability. If there is no way to improve drug solubility it will not be able to be absorbed from the gastrointestinal tract into the bloodstream and reach the site of action.

There are many ways to solubilize certain poorly soluble drugs. But these methods are limited to drugs with certain properties in regard to their chemistry (eg, solubility in certain organic media) or for example to their molecular size or conformation (eg, molecules to be incorporated into the cyclodextrin [CD] ring structure [3]). Apart from that, the usage of surfactants or cosolvents is also possible, but sometimes leads to increased side effects (eg, Cremophor EL increases the toxicity of Taxol and HP-ß-cyclodextrin is the cause of nephrotoxicity of itraconazole in Sporanox® [4]) and other disadvantages (eg, organic solvent residues). The micronization of drug powders to sizes between 1 and 10 µm in order to increase the surface area, and thus the dissolution velocity, is not sufficient to overcome bioavailability problems of many very poorly soluble drugs of the biopharmaceutical specification class II. A consequent step was to move from micronization to nanonization.

1.1 Increase of dissolution velocity by surface area enlargement

The dissolution of a solid material can be described by a diffusion layer model. This model was first suggested by Bruner and Tolloczko and further developed into its classical form by Nernst and Brunner[5].

According to the Noyes-Whitney equation, the dissolution of a material is dependent on surface area of the dissolving species:

$$\frac{dm}{dt} = \frac{DA}{h}(C_s - C),$$

where dm/dt is the dissolution rate, D is the diffusion coefficient, A is the surface area available for solvent, and h is the thickness of the diffusion layer. When $C_s >> C$, sink conditions prevail and the amount of material dissolved does not have any effect on its solubility or dissolution rate. Based on the Noyes-Whitney equation, the dissolution rate of a material can be increased by increasing the surface area available for the solvent. By moving from micronization further down to nanonization, the particle surface is further increased and thus the dissolution velocity increases too. In most cases, a low dissolution velocity is correlated with low saturation solubility.

1.2 Production of nanoparticles

There are various possibilities to produce nanoparticles in the desired shape and size. Basically three principles can be used: milling, precipitation methods and homogenization methods, as well as a combination thereof. The industrially relevant methods are the top down technologies, ie, starting from a large-size drug powder to be reduced in size. The

bottom up technologies (ie, starting from a dissolved molecule, precipitation) are currently – to our knowledge – not used in the production of commercial products. Reasons may include the need for solvent removal, the difficulty in controlling the process, and the fact that many poorly soluble drugs are poorly soluble not only in aqueous, but also organic media[6-10].

All nanocrystals in the first four products were produced using the pearl mill. Prerequisite was the availability of production facilities at sufficiently large scale. In general candidates of first choice for Nanocrystal® technology are drugs with a relatively low dose. For example, the oral single dose of Rapamune is 1 or 2 mg, the total tablet weight being approximately 365 mg for 1 mg formulation and approximately. 370 mg for the 2 mg formulation. This means it contains a very low percentage of its total weight as nanocrystals. An important point is that the drug nanocrystals are released from the tablet as ultra fi ne nanosuspension. In the event that crystal aggregation takes place to a pronounced extent, the dissolution velocity and subsequently the oral bioavailability of the BSC II drugs will be reduced. Therefore, there is an upper limit to load tablets with nanocrystals. In case the limit is exceeded and nanocrystals get in contact with each other within the excipient mixture of the tablet, the nanocrystals might fuse to larger crystals under the compression pressure during tablet production [11].

2 METHODS

2.1 Continuous flow formulation technology

Novel bottom-up nanoparticle drug delivery technology relies on controlled nano precipitation was used for the preparation of unique nanostructured drug delivery systems. The properties of the produced nanostructured particles could be modified during the process by the precise control and optimization of various reaction parameters (e.g. temperature, flow rate, pH and concentration).

NanGenex's searchable database integrated with continuous flow formulation technology enables us to predict the optimal transformation parameter for nanonization, while the integrated on-line analytics helps shorten the parameter optimization loop to reach the desired particle size. This can reduce the time needed to formulate nanostructured active molecules by as much as 90%.

Colloid solution of the nanostructured active pharmaceutical ingredients (API) was liophilized by using Scanlaf freeze dryer.

2.2 Particle size determination

Hydrodynamic diameter of the particles in colloid solution is determined by DLS-analyzer (Dynamic Light Scattering) (Nanotrac).

2.3 Physico-chemical characterization

^{1}H-NMR: The samples were measured with a Bruker DRX 300 MHz (and or) Bruker Avance II 400 MHz instrument(s). The measurements were done at room temperature (30°C) with 32 scans. Solvents were chloroform-*d* and dimethylsulfoxide-d_6. For the calibration of the spectra solvent peak and TMS signal were used in case of choroform-*d* and solvent peak was used in case of DMSO-d_6.

XRD: The structure of nanostructured particles and reference API was investigated by X-ray diffraction analysis using a Philips PW1050/1870 RTG powder-diffractometer.

Determination of saturation solubility (C_S): The solubility of nanostructured actives compared to the reference API was determined in distillate water by UV-VIS measurements (Helios Alfa UV spectrophotometer) at 292 and 296 nm wavelength and room temperature. The redispersed sample was filtered by 0.20-0.45 μm disposable syringe filter. In order to check the nanoparticle presence in the solution, it was irradiated by red laser pointer operating at 670 nm wavelength. If no scattering was observed the filtration was successful, the solution did not contain nanoparticles.

2.4 Comparative in vitro permeability tests

In vitro experiments were performed in vertical Franz-diffusion cell equipped with an autosampler (Hanson Microette TM Topical&Transdermal Diffusion Cell System, Hanson Research Corporation). During the permeability experiments 300 μl cGMP specific phosphodiesterase type 5 inhibitor solution was placed to the Prorafil membrane as donor phase. The effective diffusion surface area was 1.767 cm^2. The acceptor phase was distillate water in all cases. All measurements were performed at 37 ^{0}C and 6 parallel samples were investigated.

2.5 Comparative in vivo pharmacokinetic tests in male Sprague-Dawley rats in fasted condition

The single oral dose of reference angiotensin receptor blocker was 30 mg/kg, and that of nanostructured angiotensin receptor blocker formulation was 223.8 mg/kg

which corresponds to 30 mg/kg active agent. Both test substances were administered via gastric tube in a dosing volume of 5 ml/kg to Male Wistar rats. The vehicle of the test items was sterile 0.9% NaCl solution and the suspension was kept homogenous by continuous stirring during treatment in order to minimize the error resulting from the sedimentation. Serum samples were prepared by centrifugation (7000 rpm, 10 min, 4 ºC) of the clotted blood within 60 minutes and were stored at –20 ºC till analysis. The samples were analyzed by HPLC (Shimadzu)

3 RESULTS

3.1 Particle size of nanostructured APIs

Nanostructured nanostructured angiotensin receptor blocker and cGMP specific phosphodiesterase type 5 inhibitor were prepared by continuous flow nano precipitation technology. The produced colloid solutions were then converted into solid forms for further investigations. Both the particle size of the produced colloid solutions and redispersed nanostructured APIs in distillate water were determined by DLS technique. As it is demonstrated in Figure 1., the continuous flow nano precipitation technology resulted monodisperse nanosized active pharmaceutical ingredient which kept its nanostructured property during the solid formulation. Moreover the solid form of nanostructured APIs can be characterized by instantaneous redispersibility.

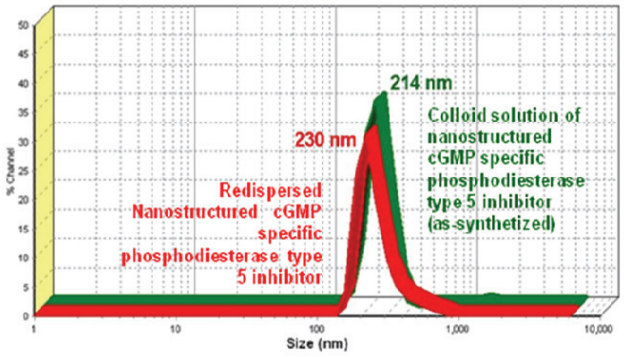

Figure 1.: Particle size and size distribution of nanostructured cGMP specific phosphodiesterase type 5 inhibitor

3.2 Physico-chemical characterization

Continuous flow nano precipitation resulted nanostructured angiotensin receptor blocker having polymorph crystal structure and partly crystalline nanostructured cGMP specific phosphodiesterase type 5 inhibitor. Chemical stability of the nanostructured APIs were investigated also. No chemical change was observed during the nanonization of active pharmaceutical ingredients, the API kept its chemical entity in the nanostructured form. The nanosized sample remained chemically stable over a 6 month test period.

3.3 Increased solubility and enhanced permeability of nanostructured cGMP specific phosphodiesterase type 5 inhibitor

Comparative solubility (C_{max}) and in vitro artificial skin permeability tests of nanosized cGMP specific phosphodiesterase type 5 inhibitor versus the reference active pharmaceutical ingredient were performed. The solubility of nanosized cGMP phosphodiesterase inhibitor was 6.8 times higher than the reference in distillate water. In in vitro experiments, the penetrated amount of nanostructured cGMP phosphodiesterase inhibitor on artificial skin was 390 % higher compared to the reference after 30 minutes of administration.

3.4 Increased solubility and enhanced permeability of nanostructured angiotensin receptor blocker

The in vivo pharmacokinetic benefit of a nanostructured angiotensin receptor blocker over the parent active molecules was also investigated. The reduction in particle size below 200 nm led to 100s fold higher solubility and 10^{3-4} fold increased drug concentration in a stable colloid solution by instantaneous redispersibility of nanosized drug (Fig.2.).

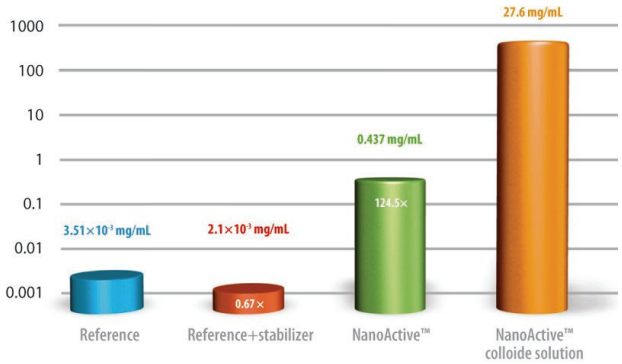

Figure 2.: Solubility enhancement of angiotensin receptor blocker by novel nanostructured drug delivery technology

The bioavailability of reference and nanostructured angiotensin receptor blocker was determined after oral administration (30 mg/kg) in fasted state in Sprague-Dawley rats using international standard protocols. Nanosized angiotensin receptor blockers had an $AUC_{15\text{-}360\ min}$ value of 6412 μg·min/ml while this value after reference treatment was 940.1 μg·min/ml. The ratio of the two AUC values ($AUC_{15\text{-}360\ min\ (nanosized)}$ / $AUC_{15\text{-}360\ min\ (reference)}$) was 6.82.

In vivo comparative pharmacokinetic tests of nanosized angiotensin receptor blocker versus reference marketed drug was also performed, the bioavailability was determined after oral administration of 30 mg/kg active ingredient in physiological saline solution at pH=5 in Sprague-Dawley rats under fed condition using international standard protocols. Nanosized angiotensin receptor blocker had an $AUC_{15\text{-}360\ min}$ value of 2744 μg·min/ml while this value after reference treatment was 1242 μg·min/ml. The ratio of the two AUC values ($AUC_{15\text{-}360\ min(nanosized)}$ / $AUC_{15\text{-}360\ min\ (reference)}$) was 2.21.

4 DISCUSSION & CONCLUSIONS

As the examples have shown, continuous flow nano precipitation technology offers great benefits. It is ideally suited for drugs with solubility problems.

Particle size reduction, unique nanostructure and the resultant increase in particle surface, curvature, saturation solubility, and consequently the increased dissolution velocity, are important factors.

Solubility enhancement alone is not the only important factor. It becomes even more important when a drug has a narrow therapeutic window where it can be absorbed. In these cases the increased solubility and dissolution velocity lead to an acceptable bioavailability.

The significant benefit that can be achieved by the unique nanostructured particle formation will be applicable to many other established drugs with limited solubility to transform them into instantaneously redispersable form with increased solubility. Moreover, the novel nanoparticle drug delivery technology enables us to design and produce nanostrucured pharmaceutical macromolecules (e.g; polypeptides, polynucleotides) and highly stable nanostructured particles for prolonged release to extend the half life of the drugs.

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