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NEW INSIGHTS IN THE DESIGN OF NANOPARTICLES ENTRAPPING DICLOFENAC

Review Article

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Abstract

Nanoparticles have a special place in nanoscience and nanotechnology, not only because of their particular properties resulting from their reduced dimensions, but also because they are promising building blocks for more complex nanostructures. Diclofenac is an anti-inflammatory drug that inhibits cyclooxygenase (COX)-2 enzyme with greater potency than COX-1 does. The adverse effects can occur when NSAIDs are used for a long time, leading to gastrointestinal and renal dysfunctions. Nanoparticles of diclofenac are nanotransporters that facilitate the intracellular penetration and can reduce the side-effects of uncorporated diclofenac. Nanoparticles incorporating diclofenac have been made using various polymers and different stabilizers. The most important parameters in the characterization of nano-systems are the particle size and their size distribution in the colloidal solution, the Zeta potential, the polymorphism, the crystallization level, the degree of drug loading and its release profile.

INTRODUCTION

In recent years, significant progress in the field of nanotechnology, especially in medicine, has been made. The medical application of nanotechnologies, usually called nanomedicine, has given an impetus decisive to the development of various types of drug transporter nano-systems, whose sizes range from 1 to 1000 nm.

In the biomedical field there are multiple types of nanotransporters, composed of different materials, such as: lipids, polymers and anorganic substances, resulting delivery systems, which, depending on their physico-chemical properties will be suitable for different applications.

Nanomedicine is currently one of the most important priorities in the European Union, with many countries calling for new plans to improve the research in this area of activity. According to its enormous potential for medicine and medical technologies, the current directions of nanomedicine are represented by the performing of the fundamental researches, consistent with the multidisciplinary vision of nanoscience and the conductions of training programs for young researchers in this field, and the specialized staff trainings for the private industry (Rakesh, Divya and Vishal, 2015).

The application of nanotechnology in obtaining the active substance delivery systems has opened a new research area in the prolonged release of various drugs. The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation. (Gelperina, Kisich, Iseman and Heifets, 2005).

NANO-TRANSPORTERS

In drug delivery technologies, nano-transporters are designed to: (i) protect a drug against the *in vivo* degradation; (ii) increase the absorption of the active substances by facilitating their diffusion into the epithelium; (iii) altering the distribution profile of the drug in the tissues, and other phases of its pharmacokinetics; (iv) facilitate the intracellular penetration and the subcellular distribution. Furthermore, the modification of the nano-transporters surface is normally used to enhance their pharmacodynamics effects. The most important results of such changes include: high stability, prolonged life of circulating nano-transporters, passive or active targeting of the affected pathological area, and increased reactivity to local stimuli, such as pH and / or changes of the local temperature (Soares, Sousa and Pais, 2018).

The nanoparticles can be made from liquid or solid lipid mixtures, which are stabilized by emulsifying substances. The lipids that are used to stabilize these nanoparticles are biocompatible and very well tolerated by the body. These types of lipids are: triglycerides, fatty acids and steroids. In addition, the use of the emulsifier combination contributes to the more efficient stabilization of such nano-systems. Lipid nanoparticles have many advantages compared to other nano-formulations, such as: facility of the large-scale production, biocompatible and biodegradable nature of the component materials, low toxic potential, the possibility of controlled and modified release of the drug, improving the solubility of the active substance as well as the possible solubility of both hydrophilic and lipophilic agents (Naseri, Valizadeh and Zakeri-Milani, 2015). Lipid nanoparticles are different from micro-emulsions, which are thermodynamically stable in lipid and water dispersion, being stabilized by surfactants and co-surfactants (Wang, Chen and Luo, 2016).

The most important parameters in the characterization of lipid nano-systems are the particle size and their size distribution in the colloidal solution, the Zeta potential, the polymorphism, the crystallization level, the degree of drug loading and its release profile. There are three different types of lipid nanoparticles: drug-homogeneous lipid matrix systems, drug-enriched core systems, and drug-enriched shell systems. The pharmacokinetics of these lipid nanoparticles entrapping drugs depends largely on the type of matrix and on the location of the active substance in the matrix; the active substance delivery from the drug-enriched shell nano-transporters presenting a higher profile. The composition of the lipid matrix, the concentration of surfactant and the manufacturing parameters, such as temperature and stirring speed, may also affect the release profiles of the drug. In essence, the idea that the most important reasons for the large-scale use of lipid nanoparticles, as an appropriate alternative to the previous polymeric nano-formulations, is the facility of large-scale production and their low toxic potential (Müller, Mäder and Gohla, 2000).

Solid lipid nanoparticles (SLNs) are the first generation of lipid-based nanocarriers that are formulated from lipids, which are solid in the body temperature and stabilized by emulsifiers (Müller et al., 2000). Nanostructured lipid carriers (NLCs) are second generation of lipid-based nanocarriers formed from mixture of solid and liquid lipids and have unstructured-matrix due to the different moieties of the constituents of NLCs (Beloqui, Solinis, Rodriguez-Gascon, Almeida and Preat, 2016).

The most important advantages of lipid nanoparticles are: the increase of large-scale

production, the biocompatible and biodegradable nature of the constituents, the possibility of controlled and modified release, the prevention of drug degradation and the maintenance of constant serum levels of the active substance. Drug-loaded lipid nanoparticles can be injected intramuscularly, intravenously, subcutaneously, but also directly into the target organs. The release of drugs from lipid nanoparticles can occur as a result of the erosion processes (such as enzymatic degradation), or by diffusion, which may explain the sustained discharge of the active substance.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

Obtaining nanoparticulate systems with targeted drug delivery is a challenge to discover agents with promising effects in regional release of the drug, especially in inflammatory and pain conditions.
Currently, non-opioid analgesics-antipyretic and non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed agents in the world.
One of the most challenging directions of research is the design and study of the modalities of incorporation of the non-steroidal anti-inflammatory drugs (NSAIDs), medicines commonly prescribed in medical practice in various pathological states accompanied by pain, fever or inflammation. The obtaining new compounds incorporating NSAIDs is of great interest, given that these drugs are of various classes, with differences in pharmacokinetic profiles, as well as with an increased risk of adverse effects in the body.
The NSAIDs are grouped into two main classes: non-selective cyclooxygenase inhibitors 1 (COX-1) and 2 (COX-2) and selective COX-2 inhibitors (Harirforoosh and Jamali, 2005). By preventing the prostaglandin (PG) biosynthesis, as a result of COX inhibition, the NSAIDs can reduce inflammation, fever and pain, which accompany various diseases. Numerous pathological processes are responsible for the development of intestinal lesions induced by the NSAIDs, especially those involving prostaglandins, mainly the E2 (PGE2) type, known to be involved in the defense processes of the digestive tract mucosa, by stabilizing the integrity of the intestinal cells. Literature data show that under inflammatory conditions and in the presence of oxidative stress the intestinal myeloperoxidase levels are increased, this parameter being considered a key biomarker for assessing the evolution of inflammation in the gastrointestinal tract.
NSAIDs are pharmaceutical active substances exerting analgesic and anti-inflammatory effects by inhibiting the cyclooxygenases (COX). NSAIDS such as naproxen, ibuprofen and diclofenac are first-line agents long-term used to treat rheumatoid arthritis and ankylosing spondylitis. The inhibition of COX-1 is responsible for the risk of severe gastrointestinal bleeding and peptic ulcer, while the inhibition of COX-2 is involved in the occurrence of cardiovascular problems. The adverse effects can occur during long-term NSAID therapy leading to gastrointestinal and renal dysfunctions. Moreover, chronic NSAID use can exacerbate a number of chronic diseases including heart failure and hypertension, and can interact with a number of drugs (eg, warfarin, corticosteroids) (Marcum and Hanlon, 2010).
Various experimental studies have shown that the use of nanoparticles entrapping NSAIDs has the advantage of significantly reducing the renal and gastrointestinal adverse effects that usually occur in the non-entrapped drug use.

Diclofenac entrapped in nanoparticles
Within the biopharmaceutics classification system (BCS), diclofenac potassium and diclofenac sodium are each BCS class II, active pharmaceutical ingredients (APIs). (Chuasuwan, Binjesoh and Polli, 2009).
It is structurally related and has similar pharmacological properties to mefenamic acid and sodium meclofenamate.
This phenylacetic acid derivative has an acidity constant of four (considered as a weak acid) and a partition coefficient of 13.4, indicating a partial solubility in both aqueous and hydrophobic environments. The structural characteristics of the molecule, namely the presence of the phenylacetic acid group and a phenyl ring containing two chlorine atoms, facilitate the maximum torsion of the phenyl ring, which provides a good fit in the coupling to the corresponding substrate binding pocket on the COX. There is evidence that diclofenac also inhibits the lipooxygenases, the phospholipase A2 and activates the anti-nociceptive pathway of the cyclic nitric oxide-guanzin monophosphate (GMP). These additional actions can explain the high potency of diclofenac, which is considered one of the strongest NSAID. The analgesic potency is six times greater than that of indomethacin, sulindac or codeine, 15 times and 40 times higher than naproxen, respectively than acetylsalicylic acid. It has a poor oral bioavailability due to its low solubility in water and in the gastric acid environment and is extensively bound to plasma albumins. It displays the enterohepatic circulation, being eliminated 35% into the bile and removed 65% from the urine. It has a relatively long duration of action (6-8 hours) but, due to the short half-life (1.5-2 hours), 3-4 daily doses are required.
In order to reduce the number of its administrations, the sustained release formulations containing diclofenac, have been developed (Schmidt, Sorensen and Pedersen, 2018). The prolonged-release forms of diclofenac sodium have been designed, in order to achieve the safety profile of the

drug and to provide a convenient once-daily administration, for the treatment of patients with chronic pain (Altman, Bosch and Brune, 2015). To avoid the adverse effects of the prolonged-release oral forms, during the time, intensive efforts to obtain nanoparticles incorporating this NDAID have been made (Patra et al., 2018).

As an amphiphilic substance, diclofenac tends to form aggregates with cellulose, which led to the imagining of different formulation techniques, to diminish its adverse effects. The incorporation of active substances with organized structures is of great interest, especially in terms of absorption and targeted action in the body.

In order to modify the pharmacokinetic profile, pharmacodynamics effects and to decrease the side effects (especially on the gastrointestinal level), several types of diclofenac nanoparticles have been designed, using various materials, such as: poly (lactide-co-glycolic acid), chitosan/poly methacrylic acid or polypyrol/menthol (Khanal et al., 2016; Cooper and Harirforoosh, 2014; Duarte Junior, Tavares and Alves, 2017; Ma et al., 2019).

It has been suggested that, the encapsulation using the phospholipids is one of the methods to improve the pharmacokinetic and pharmacodynamics aspects, but also, that the drug can induce the structural modification of phospholipids, with the development of surface active monomers (Mayuri and Mohan Chinnala, 2016).

Lipid-based formulations have been shown to significantly improve the bioavailability of hydrophobic drugs, such as diclofenac, compared to conventional dosing forms (Lopes et al., 2006). The mucus covering the surface epithelium of the gastrointestinal tract possesses a phospholipid adsorption layer, which achieves a hydrophobic area between the epithelium and the luminal content. At this level there are a number of lipid species reminiscent of active surface phospholipids, of which phosphatidylcholine is suitable to incorporate diclofenac. It was revealed that, in the presence of the ionic surfactant shield, the lipophilicity of the complex is preserved (Yamauchi et al., 2006).

Of all the nano-systems, liposomes have several advantages, represented by the low number of excipients needed for their formulation, simple preparation procedure, high physical stability and the possibility of the entrapped drug sustained release, which could be used in the treatment of various pathological conditions (Robson et al., 2018). Since 1970, liposomes have been widely used as carriers to improve the delivery of pharmacologically active substances to different target areas in the body. Liposomes are biocompatible and biodegradable. Normally, liposomes are formed from the bi-stratified phospholipids, with the hydrophilic head near the surface and the lipophilic tail away from it (Musacchio and Torchilin, 2011). Numerous experimental studies have shown that liposomes can improve therapeutic effects, but they can also reduce the adverse effects of the incorporated agents (Sercombe et al., 2015).

Various types of lipid have been used as carriers, to increase the solubility and the gastrointestinal absorption of drugs. The most commonly used is cholesterol, an ingredient that plays an important role in liposomal stabilization, increases the rigidity of the bi-layers, with improving the efficiency of encapsulation and reducing the rate of the active substance release (Abu Lila and Ishida, 2017). As the nano-carriers, liposomes have shown important biological properties such as prolonged survival in the blood, allowing the targeted attachment of ligands on surfaces, contrast properties and high sensitivity to various stimuli, which make them be excellent pharmaceutical delivery systems (Musacchio and Torchilin, 2011).

There were obtained liposomes that incorporate diclofenac with high efficiency, based on chitosan with different molecular weights, using a method of inotrope gelation, formulations that demonstrated to have significant antibacterial activity (Alqahtani, Aleanizy and El Tahir, 2019). Various studies have shown that the use of the systems of diclofenac sodium microspheres, based on sodium alginate, obtained by inotrope gelation method, has improved the *in vitro* release profile of diclofenac (Soni et al., 2013), as well as *in vivo* delivery after the oral administration in healthy volunteers (Rasel and Hasan, 2012). In the other study it was demonstrated a high degree of absorption of this non-steroidal anti-inflammatory drug incorporated in microspheres of sodium alginate/nanocristal cellulose/polyvinyl alcohol functionalized with polyethylene (Fan, Lu and Yang, 2019).

Other researchers have prepared through a thin film moisturizing technique, wide lamellar vesicles of diclofenac sodium using soy lecithin, cholesterol and ethanol solvents or a mixture of chloroform : methanol, formulations that have been characterized, the *in vitro* release profile assessed, but no *in vivo* studies have been reported (Ajith, Hyma and Tumma, 2017; Sabeti et al., 2014). Another design of nanoparticles based on the encapsulation of diclofenac sodium in phosphatidylcholine, cholesterol, stearylamine and dicethylphosphate, by a lipid-hydrating technique (Rajendar and Srinivas, 2016). These liposomes were very well characterized, the *in vitro* release curve of the entrapped drug was described, but there is no data on the effects of these nano-systems administration in laboratory animals.

Nanoparticles incorporating diclofenac have been made using various polymers and different stabilizers, such as: polyvinyl alcohol or didodecylmethylammonium bromide (Krishna and Nandini, 2016). These systems have been characterized and demonstrated an efficient

entapping and a prolonged *in vitro* release of drug, but there are no data on their *in vivo* effects.

Goh J.Z. and his collaborators obtained liposomes with diclofenac, using prolipo-Duo and dimethyl sulfoxide as solvent. It has been shown that the oral administration of these diclofenac-containing nanoparticles has produced more accentuated anti-inflammatory effects, compared to the non-entrapped drug, in carrageenan and formalin-induced paw inflammation test, respectively, in the model of experimental induced granuloma after subcutaneous pellet implantation in the rat. It has been suggested that the more intense anti-inflammatory activity of the nanoparticles with diclofenac may be due to the ability of the liposomal system to alter the bio-pharmacological properties of the entrapped drug (increasing solubilization, facilitating absorption by lymphatic transport, prolongation of the gastric transit time) and exert additional protective effects (prevention of unwanted metabolism of the substance and reduction of gastric elimination) (Goh, Tang and Zuraini, 2013).

CONCLUSIONS

Diclofenac is the most widely prescribed NSAID worldwide. Diclofenac entrapped in nanoparticles is structurally related with reducing the number of administrations, to avoid the adverse effects of the prolonged-release oral forms during time. The incorporation of active substances with organized structures is of great interest, especially in terms of absorption and targeted action in the body.

In order to modify the pharmacokinetic profile, several types of diclofenac nanoparticles have been designed, using various materials.

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