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As ciclodextrinas como potenciais agentes terapêuticos

Cyclodextrins as Potential Therapeutic Agents

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RESUMO

As ciclodextrinas (CDs) são oligossacarídeos cíclicos com especial interesse como excipientes farmacêuticos. No entanto, essas moléculas também tem se destacado como possíveis agentes terapêuticos como moduladores de lipídios e em doenças neurológicas. O tratamendo de doenças do cólon observou-se uma redução nos efeitos colaterais do fármaco causados pela administração da forma de fármaco ativo quando da utilização de complexos com CD. Os conjugados de CD também demonstraram resultados promissores no tratamento de doenças da cavidade oral e regeneração óssea local. Na área de doenças neurológicas, os CDs se destacaram porque, aparentemente, podem retardar a progressão das doenças de Alzheimer e Parkinson, reduzindo os níveis de colesterol nas membranas plasmáticas. Perspectivas futuras indicam que esses compostos não devem ser classificados apenas como excipientes, uma vez que têm atividade terapêutica em várias características doenças do século 21.

Palavras-chave: Cyclodextrinas, complexos de inclusão, conjugados, doenças neurodegenerativas. moduladores de lipídios

ABSTRACT

Cyclodextrins (CDs) are cyclic oligosaccharides with special interest as pharmaceutical excipients. However, these molecules have also come to stand out as potential therapeutic agents in drug targeting and as lipid modulators in neurological diseases. Colon targeting through the formation of CD conjugates revealed no changes in the therapeutic efficacy, however, a reduction in drug side effects caused by the active drug form administration was observed. CD conjugates also demonstrated promising results in the treatment of diseases of the oral cavity and local bone regeneration. In the area of neurological diseases, CDs stood out because apparently they can slow the progression of Alzheimer's and Parkinson's diseases by lowering the cholesterol levels in the plasma membranes. Future perspectives indicate that these compounds should not be classified only as simple excipients, once they have therapeutic activity in several diseases characteristics of the 21th century.

Keywords: Cyclodextrins, inclusion complexes, conjugates, drug targeting, lipid modulators, neurodegenerative diseases.

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INTRODUÇÃO

Cyclodextrins are cyclic oligosaccharides coming from starch which were described first by Villier in 1891 (VEIGA, PECORELLI & RIBEIRO, 2006; VEIGA & FIGUEIRAS, 2011).

The unique structure of these molecules is the main responsible for the wide use of cyclodextrins in several industries, namely in pharmaceutical industry (DUCHÊNE, 2011).

Their ability to encapsulate drugs, resulting in inclusion complexes, can solve some problems related to their properties, namely unwanted organoleptic characteristics (OLIVEIRA, SANTOS & COELHO, 2009), weak aqueous solubility and instability (CUNHA-FILHO & SÁ-BARRETO, 2007).

CDs have been extensively used in investigation and development of several pharmaceutical formulations and currently diverse CDs are included in formulations all around the world (CARRIER, MILLER & AHMED, 2007).

More than 100 years after their discovery, their versatility and their multifunctional characteristics are responsible for the continuous discover of new cyclodextrins technology-based. These molecules are a new excipient with enormous potential (VEIGA & FIGUEIRAS, 2011).

Recently, CDs have been indicated as potential therapeutic agents in different areas, namely drug targeting (YANO et al., 2014; LIU et al., 2007; LIU et al., 2008; VADNERKAR & DHANESHWAR, 2013) and as lipid modulators in neurologic diseases (WOOD et al., 2003; RECCHIA et al., 2004; QINGHUA, LI & SENFANG, 2006; YAO et al., 2012).

Several studies tried to deliver the drug at a specific site of action trough the formation and administration of CDs conjugates (YANO et al., 2014; LIU et al., 2007; LIU et al., 2008; VADNERKAR & DHANESHWAR, 2013) We highlight colon-targeting, which exploits the protection that CD conjugates give the drug, allowing it to reach the therapeutic target, i.e. the end of the intestine, unchanged (YANO et al., 2014; VADNERKAR & DHANESHWAR, 2013). Also, in the development of formulations to treat oral cavity pathologies (LIU et al., 2007) and repairing bone damage, attempts have been performed in order to override the obstacles resorting to the use of these conjugates (LIU et al., 2008).

In neurological diseases, CDs application was driven by their ability to extract cholesterol (DREYFUSS & OPPENHEIMER, 2011) from the plasma membranes, considering that high levels of this lipid were associated to an higher increase of neurodegeneration in several studies (RECCHIA et al., 2004; SHOBAB, HSIUNG & FELDMAN, 2005).

The main purpose of this review is to show the versatility of these molecules as potential therapeutic agents. Thus, after approaching the structure and physicochemical properties of CDs, their above-mentioned therapeutic applications are described and exemplified in detail.

1. Origin, attainment and structure of cyclodextrins

CDs are natural compounds, obtained from the hydrolysis of starch by enzyme action of cyclodextrin glycosyltransferase (CGTase) (LOFTSSON & BREWSTER, 1996; VEIGA, PECORELLI & RIBEIRO, 2006; DUCHÊNE, 2011). These are cyclic oligosaccharides, composed by D-glucopyranose monomers (glucose) bound together through α-1,4 glycosidic bonds.(5, 18) Due to the absence of free rotation of the glycosidic bounds and to the chain conformation of the glucose units, CDs present a cylindrical-conical shape (LOFTSSON & BREWSTER, 1996; OLIVEIRA, SANTOS & COELHO, 2009).

In their structure, CDs present externally located hydroxyl groups, which give them hydrophilic characteristics, making them water soluble and insoluble in most organic solvents.(4, 5) Secondary hydroxyls are bound to the carbon atoms located in position 2 and 3 (C2 and C3) of the glucose monomers, in the largest extremity, and the primary hydroxyl groups bound to the carbon atoms in position 6 (C6) of the glucose units, in the narrowest opposite end. This structure is due to the free rotation of the primary hydroxyl groups, which narrows the effective diameter of the cavity in the narrowest end, against the secondary hydroxyls that do not have that rotating movement (VEIGA, PECORELLI & RIBEIRO, 2006). The inside of the cavity is constituted by two rings of C-H groups (in C3 and C5) and by a ring of glycosidic oxygen atoms, giving this face hydrophobic characteristics (OLIVEIRA, SANTOS & COELHO, 2009).

Figure 1 - Structure and size of the natural CDs cavity.
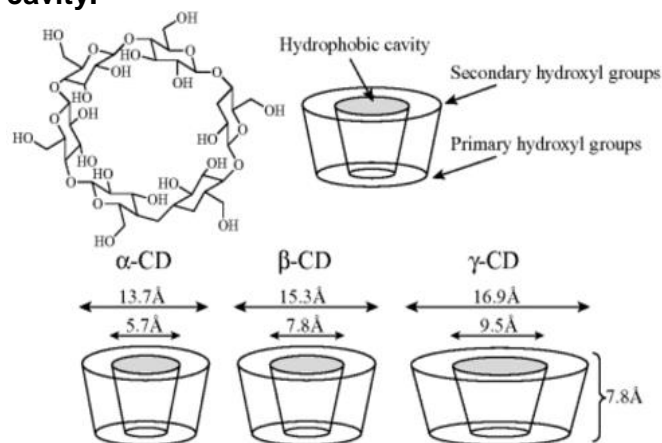
Legend: Adapted from AHUJA, et al., 2011.

The enzyme responsible for the hydrolysis of some glycoside starch bounds, with consequent formation of dextrins, is present in various microorganisms species, such as *Bacillus macerans* and *B. circulans* (VEIGA, PECORELLI & RIBEIRO, 2006; DUCHÊNE, 2011). CGTase also has the ability to promote the cyclization of the oligosaccharides fragments resulting from the hydrolysis, originating cyclic products - called cyclodextrins (VEIGA & FIGUEIRAS, 2011).

The main steps of the CD production process are:

- Culture of microorganism that produces the CGTase enzyme;
- Separation of the enzyme, followed by its concentration and purification;
- Enzyme conversion of pre-hydrolysed starch (mixture of acyclic dextrins), into a mixture of cyclic and acyclic dextrins;
- Separation of CDs from the previously referred mixture, their purification and crystallization (VEIGA & FIGUEIRAS, 2011).

Depending on the microorganism that produces the enzyme and the exact conditions of the reaction, there are three natural CDs, i.e., those that are obtained in higher percentage: α-CD, β-CD and γ-CD, which have 6, 7 and 8 glucose units, respectively (UEKAMA, HIRAYAMA & IRIE, 1998; CUNHA-FILHO & SÁ-BARRETO, 2007; DUCHÊNE, 2011).

CDs with more than 8 units are difficult to isolate, have weak capacity of complexation and, therefore, have reduced pharmaceutical interest. Possibly due to steric reasons there are no CDs with less than 6 glucose units

As a consequence of the different number of glucose units, CDs present different properties in accordance with what is indicated in table I (CHALLA et al., 2005; DUCHÊNE, 2011).

Table I - Physicochemical properties of α-CD, β-CD and γ-CD.

Type of CD	α	β	γ
Glucose units (no.)	6	7	8
Molecular mass (Da)	972	1135	1297
Aqueous solubility (g/100ml at 25ºC)	14.4	1.85	23.2
Internal cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Height of the cylindrical-conical structure (Å)	7.9	7.9	7.9
Approximate cavity volume (Å)	174	262	472

Legend: Adapted from AHUJA, et al., 2011.

β-CD is the one that presents lowest solubility, because its structure favours the formation of a high number of intra-molecular hydrogen bounds between the existing secondary hydroxyl groups, making its hydration by water molecules difficult and making its structure rigid (VEIGA & FIGUEIRAS, 2011; DUCHÊNE, 2011).

This phenomenon is not seen in α-CD, where the glucose molecules are found in distorted position, allowing only four of the six possible hydrogen bounds to establish (VEIGA & FIGUEIRAS, 2011).

γ-CD is the most soluble of the three natural CD, because it is no coplanar molecule and it presents a more flexible structure (VEIGA & FIGUEIRAS, 2011).

β-CD has been widely used in several pharmaceutical applications due to its immediate bioavailability in high quantities and at a reduced cost (VEIGA & FIGUEIRAS, 2011), but mainly because its cavity has an adequate size in the complexation of a wide range of drugs (CHALLA et al., 2005; VEIGA, PECORELLI & RIBEIRO, 2006). This CD seems to be the most useful in the pharmaceutical sector, fact proven by its presence in more than half the pharmaceutical formulations with CDs available in market (VEIGA & FIGUEIRAS, 2011).

However, this cyclodextrin also has disadvantages, namely, high nephrotoxicity, due to its low aqueous solubility. This reality limits its therapeutic use, mainly dealing with formulations for parenteral administration (CHALLA et al., 2005;

VEIGA, PECORELLI & RIBEIRO, 2006; CUNHA-FILHO & SÁ-BARRETO, 2007).

2. Formation of inclusion complexes

Drugs rarely exhibit the desired physicochemical properties and at the time of developing the pharmaceutical formulation that will include the therapeutic agent, many difficulties may arise. Among the most common, there is the low aqueous solubility of the drug, its bitter taste, the drug being irritant to the mucous membranes or target tissues, being susceptible of destruction factors, being volatile, among others (VYAS, SARAF & SARAF, 2008).

The unique structure that CDs present, gives them the ability to encapsulate in their cavity a large variety of molecules and, therefore, overcome the above-mentioned problems (VYAS, SARAF & SARAF, 2008). CDs structure, namely the presence of the cavity, allows the total or partial inclusion of most of the hydrophobic drugs, either in solution either solid, and the formed complexes are called inclusion complexes (CI) (VEIGA, PECORELLI & RIBEIRO, 2006; VEIGA & FIGUEIRAS, 2011; DUCHÊNE, 2011).

This is one of the CDs properties, mostly responsible for their wide use in a wide variety of industries (DUCHÊNE, 2011).

In the formation of the inclusion complexes, the encapsulated or "guest" molecule is surrounded in the characteristic hydrophobic environment of the CD or "host" cavity (VEIGA & FIGUEIRAS, 2011). Thus, the essential criterion to form these is that the included molecule has the adequate size and shape, so that it can fit the cavity of the CD, being totally or partially encapsulated (VEIGA, PECORELLI & RIBEIRO, 2006; VENTURINI et al., 2008; OLIVEIRA, SANTOS & COELHO, 2009).

The CD cavity has a higher affinity to lodge apolar compounds and preferably subtracts in their neutral form (VEIGA & FIGUEIRAS, 2011) The CI formation process is conditioned by several factors, namely by the structure and physicochemical properties, either of the encapsulated drugs, either of CDs (OLIVEIRA, SANTOS & COELHO, 2009; VEIGA & FIGUEIRAS, 2011).

The size of the cavity is one of the factors that determines the ability of CD complexation.(2) This should be sufficient to include the molecule, but not too much, being at risk of being inadequate to establish guest-CD interactions (DUCHÊNE, 2011). α-CD may be useful in the inclusion of small size molecules or lateral chains of large molecules, in contrast, γ-CD allows to complex considerable size molecules. β-CD, in turn, is adequate to complex aromatic rings (VEIGA & FIGUEIRAS, 2011).

Molecules with structure higher than the CDs cavity do not necessarily have to be excluded. The formation of CI with those molecules is possible, as long as they have lateral chains adequate to suffer partial inclusion (VYAS, SARAF & SARAF, 2008; VEIGA & FIGUEIRAS, 2011).

It should also be highlighted that, depending on the size of the guest molecule and of the CD, a molecule may interact with more than one CD, as well as a CD may interact with more than one guest molecule (DUCHÊNE, 2011).

The mechanism of formation of these complexes covers several steps, among which are included: the approach between the substratum and the CD, the desolvation of the substrate and of the CD internal cavity, receptor-substrate interactions and, finally, a reorganization of the solvent around and inside the cavity (VEIGA, PECORELLI & RIBEIRO, 2006; VENTURINI et al., 2008).

As the CDs cavity is apolar, the water molecules inside are in an energetically unfavourable state, given the nature of the polar-apolar interaction (LOFTSSON & BREWSTER, 1996; VENTURINI et al., 2008). Thus, these water molecules can be easily replaced by guest molecules with a lower polarity degree (VEIGA & FIGUEIRAS, 2011; VENTURINI et al., 2008). The replacement of high enthalpy water molecules by adequate substrate, i.e., less polar, ending in a decrease of the system energy and therefore in its higher stability, is considered the main driving force for the complexation (VEIGA & FIGUEIRAS, 2011; LOFTSSON & BREWSTER, 1996; VENTURINI et al., 2008).

In this process there is no establishment of covalent bounds, which allows the complexes to be easily dissociated in physiological conditions. However, Van der Waals interactions, hydrogen bounds, hydrophobic interactions and changes of the superficial tension of the solvent may participate (VEIGA & FIGUEIRAS, 2011).

After formation of CI, the physicochemical properties of the complexed drug and CD are changed, being this the main principle to confirm their formation (CUNHA-FILHO & SÁ-BARRETO, 2007).

The stability of the complex, in aqueous solution, is reflected in the dynamic balance between the free drug molecules and the complexed molecules, and is characterised by the constant of complexation (Kc), also known as constant of association or stability (VEIGA &

FIGUEIRAS, 2011; OLIVEIRA, SANTOS & COELHO, 2009). This constant depends on the ability of fitting of the guest molecule in the CD cavity and can be quantified by the equation (I), where [Drug-CD], [Drug] and [CD] represent the complexed drug, free drug and free CD concentration, respectively (VEIGA, PECORELLI & RIBEIRO, 2006; VEIGA & FIGUEIRAS, 2011; DUCHÊNE, 2011).

$$K_c = \frac{[Drug-CD]}{[Drug][CD]}$$ Equation (I)

The dissociation kinetics of the complex is related to Kc, being the value of this the higher the more stable CI is and therefore the lower the dissociation degree (OLIVEIRA, SANTOS & COELHO, 2009; VEIGA & FIGUEIRAS, 2011). Generally, the force of the complex is proportional to the structural complementarity between the encapsulated molecule and the CD cavity (DUCHÊNE, 2011).

Being the complexation a dynamic process, the complexes are always being formed and dissociated, and their half-life time is only a few milliseconds, even when they have high Kc values (VEIGA & FIGUEIRAS, 2011). Thus, kinetics of drug release from the inclusion complex is not a limiting factor in its absorption (OLIVEIRA, SANTOS & COELHO, 2009; VEIGA & FIGUEIRAS, 2011).

Figure 2 - Dynamic balance of an inclusion complex.
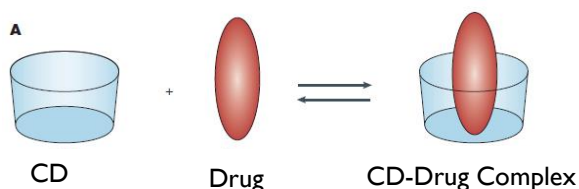
Legend: Adapted from OLIVEIRA & COELHO, 2009.

The dilution effect appears as the main responsible for the dissociation of the inclusion complexes, being more prominent when the administration is made orally or parenterally, since both have a high volume of dilution (STELLA et al., 1999; LOFTSSON & MASSON, 2001; VEIGA & FIGUEIRAS, 2011).

When the stability of the complexes is moderate or weak, the dilution effect may be enough to release the drug quickly and totally. However, there are situations in which the contribution of other factors to release the drug from CDs complexes should be considered, namely when resorting to administration routes with lower dilution volume (ocular or transdermal use), as well as when there are strong drug-CD bounds (LOFTSSON & MASSON, 1999; STELLA et al., 1999; VEIGA & FIGUEIRAS, 2011).

In these cases, the complex dissociation may be aided by the bound of the drug to the plasma proteins or to tissues proteins and by the competitive replacement of the encapsulated drug by endogenous compounds (bile salts, cholesterol, skin lipids) (VEIGA & FIGUEIRAS, 2011; DUCHÊNE, 2011). The principle is based on the simple transfer of the drug into matrices with which it has a higher affinity (LOFTSSON & MASSON, 1996; VEIGA & FIGUEIRAS, 2011).

In the presence of ionisable drugs or CDs, dissolution may be achieved by the exposure of the complex to a pH that changes the state of ionization, together with an increase in the solubility and a decrease of Kc (VEIGA & FIGUEIRAS, 2011).

The formation of the inclusion complexes is an exothermic process, as such the increase of the temperature may culminate in a decrease of its stability, also contributing to increase the fraction of free drug (LOFTSSON & MASSON, 2001; VEIGA & FIGUEIRAS, 2011)

The contribution of each one of the above-mentioned mechanisms is not tight, it changes with the route of administration, the volume of distribution of the drug and CD, the Kc value of the complex, the drug, CD and proteins concentrations and the presence of possible competitive agents (STELLA et al., 1999).

3. Cyclodextrins derivatives

Even though natural CDs appear as fundamental tools to overcome several problems associated with the investigation and development of pharmaceutical formulations, these present a few limitations as drugs vehicles (VEIGA, PECORELLI & RIBEIRO, 2006; VEIGA & FIGUEIRAS, 2011).

With the purpose of improving the physicochemical properties, the ability of inclusion (VEIGA, PECORELLI & RIBEIRO, 2006; VEIGA & FIGUEIRAS, 2011; DUCHÊNE, 2011) and minimizing the parenteral toxicity (VEIGA, PECORELLI & RIBEIRO, 2006) associated with natural CDs, chemical modified CDs appeared (VYAS, SARAF & SARAF, 2008).

The main cause of the low solubility of natural CDs is the formation of hydrogen bounds between the hydroxyl groups of its structure (LOFTSSON & BREWSTER, 1996). The introduction of different substituents, even with hydrophobic characteristics (VEIGA & FIGUEIRAS, 2011), in the primary and/or

secondary hydroxyl groups, results in an impediment of the establishment of such bounds, allowing the considerable increase of the CD solubility (LOFTSSON & BREWSTER, 1996). These changes in the chemical structure may also result in molecules with a lower crystalline organization (KURKOV & LOFTSSON, 2013) or amorphous, being other of the factors that contributes to the increase of their solubility (VEIGA & FIGUEIRAS, 2011).

The derivatives may be obtained by replacement of the hydroxyl groups by methyl, ethyl, carboxymethyl, hydroxyethyl, hydroxypropyl, saccharide groups or through CDs polymerization (VEIGA & FIGUEIRAS, 2011). The substituents may or may not be bound to the same glucose monomer (VARCA et al., 2010).

As a result of the introduced substituents, the chemical derivatives of CDs were rated in three huge groups: hydrophilic, hydrophobic and ionisable (VYAS, SARAF & SARAF, 2008; BARRETO & CUNHA-FILHO, 2008).

The derivatives may present an improvement of their properties regarding natural CDs, in what concerns their solubility, toxicity, complexation ability and stability (VARCA et al., 2010).

These are also highlighted by their behaviour as drugs delivery systems, being therefore capable of improving the bioavailability of the encapsulated molecule, as well as to control its activity (VARCA et al., 2010).

Hydrophilic derivatives, where methylated, hydroxyalkylated (BARRETO & CUNHA-FILHO, 2008) and ramified CDs (hydroxyl groups replaced by mono and disaccharides) are included, present a aqueous solubility higher than the one of the original molecule (VEIGA, PECORELLI & RIBEIRO, 2006). On the contrary, hydrophobic CDs, as acylated and ethylated derivatives, have a lower solubility (VEIGA, PECORELLI & RIBEIRO, 2006).

Hydrophilic derivatives improve the aqueous solubility and the dissolution rate of drugs with low solubility in water, being useful to improve the absorption through biological barriers (VYAS, SARAF & SARAF, 2008), while hydrophobic derivatives delay the dissolution rate of water-soluble drugs (CHALLA et al., 2005).

Due to their characteristics, hydrophilic CDs may be used as immediate drug release systems, instead of hydrophobic derivatives which reveal useful when it is intended to obtain prolonged release formulations (CHALLA et al., 2005; VYAS, SARAF & SARAF, 2008).

Ionisable derivatives, on the other hand, are capable of being used to obtain delayed release formulations, being one of them 6-O-(carboxymethyl)-O-ethyl-β-CD (CME-β-CD) (CHALLA et al., 2005).

CME-β-CD has pH dependent solubility, this means that the solubility of the complex is limited in the acidic conditions of the stomach, increasing with the increase of the pH along the gastrointestinal tract (GIT) (CHALLA et al., 2005). In front of this characteristic, a selective dissolution of the CD-drug complex is possible, being CME-β-CD useful in the development of enteric formulations (CHALLA et al., 2005; VYAS, SARAF & SARAF, 2008).

Summarising, both natural CDs and their chemical derivatives are useful in the development of formulations, due to their ability to improve solubility, dissolution rate, chemical stability and drugs absorption (AHUJA et al., 2011).

Despite the wide range of existing CDs derivatives, most of these molecules do not have practical application due to the complex and expensive synthesis process inherent to them (VEIGA & FIGUEIRAS, 2011).

4. Potential therapeutic applications of cyclodextrins

In pharmaceutical technology, CDs may be useful in changing physicochemical properties of the compounds, stability, chemical reactivity and also in changing biopharmaceutical properties (VEIGA & FIGUEIRAS, 2011).

These molecules have an important role in the development of formulations containing drugs with low aqueous solubility, allowing to improve its apparent solubility and dissolution. Such aspect is achieved through the formation of CIs or solid dispersions where CDs act as hydrophilic transporters to compounds with inadequate characteristics for complexation. Such fact also allows to improve permeability due to the increase of the drug quantity together with the biological membrane available to suffer absorption, which translates into an increase of its bioavailability.

The solubility increase also allows to minimize the toxicity and the appearance of side effects, as it makes the drug effective when administered in lower doses (CHALLA et al., 2005).

Beyond their conventional applications as pharmaceutical excipient, CDs have revealed applicability as potential therapeutic agents. Next, some of these applications will be approached and exemplified in detail.

5.1. Vectors to therapeutic targets by formation of conjugates

In the pharmaceutical sector, the ideal formulation should be capable of directing the drug specifically into the site of action (SALMASO & SONVICO, 2011), in the quantity and period of time needed, minimizing its side effects and optimizing its therapeutic effects (CHALLA et al., 2005).

In the last years, CDs have been seen as more than simple excipients, giving special emphasis to their application as multifunction carriers (SALMASO & SONVICO, 2011).

To some applications, namely drug targeting, the dynamic balance association-dissociation of CI is not desirable (BARRETO & CUNHA-FILHO, 2008), mainly when the complex is dissociated before reaching the target site where the drug should be delivered (VYAS, SARAF & SARAF, 2008; BARRETO & CUNHA-FILHO, 2008; VEIGA, FIGUEIRAS & VIEIRA, 2011).

This limitation may be overcome through the establishment of covalent bounds between the drug and CD, originating the called conjugates VYAS, SARAF & SARAF, 2008; VEIGA, FIGUEIRAS & VIEIRA, 2011).

CDs when orally administered are scarcely metabolised along GIT, because their cyclic form leaves unavailable the terminal groups susceptible of salivary and pancreatic enzymatic hydrolysis (BARRETO & CUNHA-FILHO, 2008). These facts together with the high size of these molecules (VEIGA & FIGUEIRAS, 2011) are responsible for their reduced absorption, on the stomach and on the small intestine (UEKAMA, HIRAYAMA & IRIE, 1998; VYAS, SARAF & SARAF, 2008).

Despite travelling GIT practically intact, it is when reaching the large intestine that they find the site where their degradation takes place (CHALLA et al., 2005). CDs suffer fermentation by the bacterial flora of the colon (VADNERKAR & DHANESHWAR, 2013) originating small saccharides, being these then absorbed as maltose and glucose (VYAS, SARAF & SARAF, 2008). These saccharides may also be metabolised and, finally, excreted (AHUJA et al., 2011).

The unique metabolism of CDs thus elucidates the fact that the colon is the GIT site that most interest raises in drug targeting resorting to these molecules (VYAS, SARAF & SARAF, 2008; AHUJA et al., 2011; SALMASO & SONVICO, 2011). This vectorization is especially attractive when the purpose is to deliver drugs for the treatment of local pathologies, as colon cancer and/or inflammatory bowel disease (MINAMI, HIRAYAMA & UEKAMA, 1998; SALMASO & SONVICO, 2011; DHANESHWAR & VADNERKAR, 2011).

The release and absorption of peptides and proteins (FRIEND, 1998) and other drugs that suffer degradation in the upper GIT (MINAMI, HIRAYAMA & UEKAMA, 1998; SALMASO & SONVICO, 2011; DHANESHWAR & VADNERKAR, 2011), as well as the release of vaccines, are other of the potential applications that this target site has available (DHANESHWAR & VADNERKAR, 2011).

This versatility of the colon is enabled by the physiological and chemical properties that it gathers, namely pH near neutral, long transit time and relatively low enzyme activity (SALMASO & SONVICO, 2011).

Several studies have resorted to the synthesis of pro-drugs using CDs in the attempt to reach a site-selective drug release having the colon as therapeutic target (MINAMI, HIRAYAMA & UEKAMA, 1998; SALMASO & SONVICO, 2011).

Summarising, targeting of colon through pro-drugs implies the formation of conjugates between the drug and cyclodextrin that resist the passage through the upper GIT, but that are degraded by enzymes of the colon microflora, resulting in the release of the active drug and its consequent absorption (DHANESHWAR & VADNERKAR, 2011; VADNERKAR & DHANESHWAR, 2013).

Inflammatory bowel disease is an idiopathic chronic inflammatory pathology which affects the colon mucosa and submucosa (PODOLSKY, 1991).

Current therapy of this disease has not yet reached healing, focusing on the use of drugs that minimize inflammation (MEISSNER & LAMPRECHT, 2008). Among these, there is sulphasalazine, 4-aminosalicylic acid and the class of corticosteroids, where prednisolone is included (RHODES, THOMAS & EVANS, 1997; SALMASO & SONVICO, 2011).

Prednisolone, when orally administered, suffers partial absorption in the upper GIT, ending in a decrease of the quantity of drug that reaches the colon and therefore of its therapeutic efficacy, at the same time triggering the appearance of systemic side effects (FRIEND, 1998; SALMASO & SONVICO, 2011).

In the attempt to overcome this obstacle, a study was performed in which changes in the anti-inflammatory effect and in the side effects of prednisolone were evaluated and compared, using two distinct pharmaceutical formulations:

prednisolone and the α-CD with prednisolone conjugate, which was obtained from the formation of a ester bound (YANO, 2002).

The authors concluded that the anti-inflammatory effect was comparable in both formulations, however the side effects resulting from the administration of α-CD-prednisolone conjugate were lower. These results were supported by the reduction of the degradation of the complex in the stomach and small intestine, minimizing its systemic absorption and therefore the appearance of undesirable effects (YANO, 2002).

The α-CD-prednisolone conjugate reveals advantageous because it allows a reduction of the drug side effects, without prejudice of its anti-inflammatory activity (YANO, 2002; VYAS, SARAF & SARAF, 2008).

Figure 3 - Proposed mechanism for the anti-inflammatory effect and for the decrease of side effects after oral administration of PD (A) and of the α-CD-PD conjugate (B).
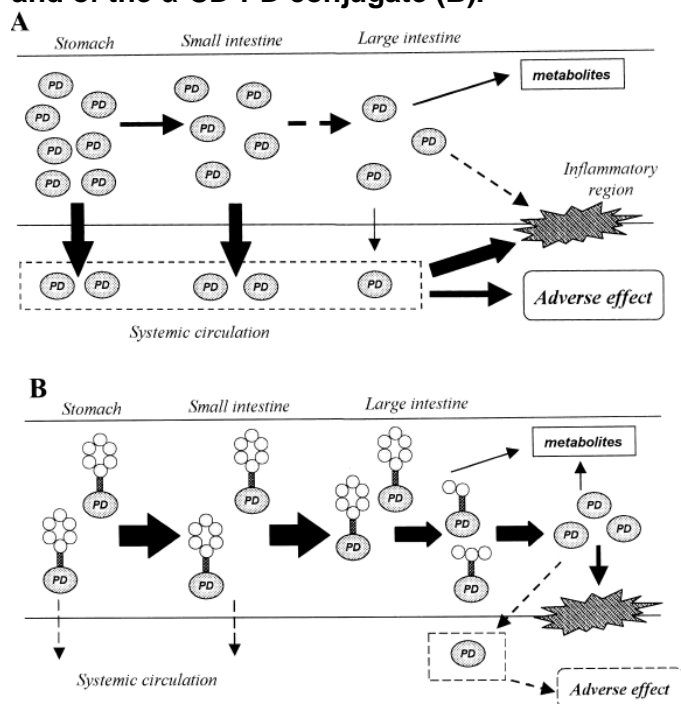
Legend: Adapted from YANO, 2002.

Recently, a study with 4-aminosalicylic acid, a drug that presents promising activity in the treatment of inflammatory bowel disease, but that when orally administered, does not reach effective concentrations in the colon and triggers side effects, as GIT irritation, was developed (VADNERKAR & DHANESHWAR, 2013).

In this investigation, a 4-aminosalicylic acid-β-CD conjugate was prepared, which was then orally administrated, to rats with induced colitis. The purpose of this study was to evaluate the therapeutic effect of the conjugate, as well as the appearance of side effects. The results were confronted with other animal groups, in which different drugs were administered to treat the disease: 1) sulphasalazine, 2) 5-aminosalicylic acid and 3) 4-aminosalicylic acid.

The obtained results showed that the administration of the conjugate did not reveal improvement of the therapeutic effect, however this highlighted due to its safety profile.

The significant reduction in the risk of ulcer induction, therefore minimizing the toxicity in the upper GIT, allowed the 4-aminosalicylic acid-β-CD conjugate to preserve its potential as therapeutic agent in the treatment of inflammatory bowel disease when compared with the remaining tested drugs (VADNERKAR & DHANESHWAR, 2013).

Other studies performed have disclosed new applications of the CDs conjugates, namely in the treatment of oral cavity pathologies (LIU et al., 2007) and in local bone regeneration (LIU et al., 2008).

The development of formulations to treat oral cavity pathologies presents some difficulties, among which the drug retention in the lesion, in the adequate quantities and for the period of time needed to reach therapeutic efficacy and minimise the appearance of side effects (LIU et al., 2007).

The following study explored a new mechanism of retention, whose basic principal lied on the development of a drug delivery system that bound to dental enamel, to ease the local distribution of the drug to the surrounding soft tissues. Diseases that may be treated by this mechanism are, therefore, contained to the areas around enamel, as periodontitis (LIU et al., 2007).

Bisphosphonates are a class of drugs, in which alendronate (ALN) is included, and that have shown to have strong tropism to the bone tissue (LIN, 1996; LIU et al., 2007). Due to this characteristic and to the availability of a NH2 group for conjugation, the authors developed a conjugate between alendronate and β-CD.(10) This CD, on its hand, was selected due to its ability to form CIs with drug molecules (LIU et al., 2007; VYAS, SARAF & SARAF, 2008).

The conjugate kept the osteothropic characteristics, as well as the ability to encapsulate compounds. The latter was evaluated through the formation of complexes with dexamethasone (Dex), a drug used in the treatment of oral cavity pathologies molecules (LIU et al., 2007).

When the conjugate is bound to hydroxyapatite (HA), main component of dental enamel and bones (LIU et al., 2008), the drug release becomes proportional to the quantity of

saliva secreted, due to the dilution effect (LIU et al., 2007).

The authors of the study were able to develop a new delivery system of drugs derivative from β-CD (ALN-β-CD), which presents a strong bound to HA, allowing it to target the drug, and that may form complexes with different therapeutic agents, as Dex. Potentially, this system may have interest in the development of topical formulations and in the clinical treatment of a wide variety of oral cavity pathologies (LIU et al., 2007).

Osteothropism of ALN-β-CD conjugate also supported its application in local bone regeneration studies (LIU et al., 2007). Currently the repair of bone damages is invasive and costly, so the current investigation has sought new solutions.

Prostaglandins (PGs) owe their notoriety mainly to the role that they perform in the inflammatory process, however they are relevant in other contexts, namely as anabolic agents of the bone structure (VROTSOS, MILLER & MARKS, 2003). Despite that, these molecules when directly administered cause side effects in the soft tissues, which motivated the development of a complex between the ALN-β-CD conjugate and PG E. (LIU et al., 2008).

The results showed that ALN-β-CD/PG E complex induced a very strong anabolic reaction in the bone area where it was administered. However, the administration of only the ALN-β-CD conjugate originated a higher bone formation (LIU et al., 2008).

The authors of this study accidentally found out that the ALN-β-CD conjugate had anabolic effect, without the side effects associated to PGs (LIU et al., 2008; SALMASO & SONVICO, 2011). Even though the mechanism is not completely highlighted, it is though that it results from the integration of ALN and β-CD characteristics. While the CD cavity allows the complexation of endogenous anabolic compounds (PG E, lipids, steroids, cholesterol, vitamin D, etc.), ALN mediates the fixation to the bone surface (LIU et al., 2008).

The conjugate revealed to be a potential therapeutic agent in repairing bone defects, however more studies are needed to validate this new therapeutic application (LIU et al., 2008).

The assignment of the name therapeutic agent to CDs is controversial, namely because they work mostly as carriers in drug targeting, having no direct therapeutic effect. Despite that, this should not be excluded, because without the help of these molecules the therapeutic target would not be reached, or would be reached in less favourable conditions. Thus, the CDs importance

in this area of pharmaceutical technology is well marked.

5.2. Potential lipid modulators in neurological diseases

Neurological diseases are a relevant branch of scientific investigation, where Alzheimer's Disease (AD) and Parkinson's Disease (PD) are included. Despite being two distinct clinicopathological entities, these two neurodegenerative disorders are the main causes of dementia in the elderly and are characterised by an anatomic or physiological, progressive and selective loss, related to neural systems (ESPOSITO & CUZZOCREA, 2010; SERRANO-POZO et al., 2011).

The lack of drugs that change the course of these diseases is the main fact that triggered the investigation of new therapies for their treatment, or preferentially, for their prevention (ESPOSITO & CUZZOCREA, 2010).

5.2.1. Alzheimer's Disease

Aetiology of AD is not fully highlighted (SHOBAB, HSIUNG & FELDMAN, 2005), but it is known that only a small number of cases are due to genetic mutations (YIP et al., 2001).

Pathophysiology of it is complex and involves multiple interconnected pathways (SCHMITT et al., 2004). However, the formation of amyloid plates in the brain is considered the main responsible for the neural death in this pathology (SCHMITT et al., 2004; RITTER, 2012).

These plates consist in insoluble aggregates of different isoforms (39-42 amino acids) of β-amyloid protein (βA) (YIP et al., 2001; SCHMITT et al., 2004; ESPOSITO & CUZZOCREA, 2010; SERRANO-POZO et al., 2011; ABRAMOV et al., 2011). Deposition of this protein has been assigned to several factors, namely an increase of the βA protein concentration, its specific interactions with proteins/lipids promoter of the aggregation and/or inability to eliminate βA from the brain parenchyma (YIP et al., 2001).

The neurotoxic βA peptide is a product that results from the enzyme cleavage, by secretases (SHOBAB, HSIUNG & FELDMAN, 2005), of the amyloid precursor protein (APP), a normal component of the cellular membrane of the healthy neurons (RITTER, 2012), with both proteins located in the lipid rafts (ECKERT et al., 2010). Its production is conditioned by the cholesterol availability in the nerve cells membranes (SCHMITT et al., 2004), which may modulate the

secretase activity (ECKERT et al., 2010).

The high content of cholesterol in the lipid rafts, areas of the plasma membrane where secretases are, eases the bound of β- and γ-secretase to their substrates in an adequate configuration to promote an APP unwanted pathogenic cleavage (SHOBAB, HSIUNG & FELDMAN, 2005). Thus, high cholesterol cell levels lead to the increase of the process of plates formation, by contributing to the production of amyloidogenic isoforms - βA-40 and βA-42 (SCHMITT et al., 2004; RITTER, 2012).

On the contrary, low levels lead to the increase of the APP physiological metabolism by α-secretase originating a non-amyloidogenic precursor or soluble APP (sAPP) (SCHMITT et al., 2004; RITTER, 2012).

Considering the previously referred information, cholesterol depletion from neural cells appears as a viable therapeutic approach in the treatment of AD (SCHMITT et al., 2004). As a consequence, the peculiar structure of CDs, which allows the inclusion of hydrophobic compounds in the lipophilic cavity, propelled their use in several studies of this nature (BAR-ON et al., 2006).

Figure 4 - Formation of amyloid plates in AD.
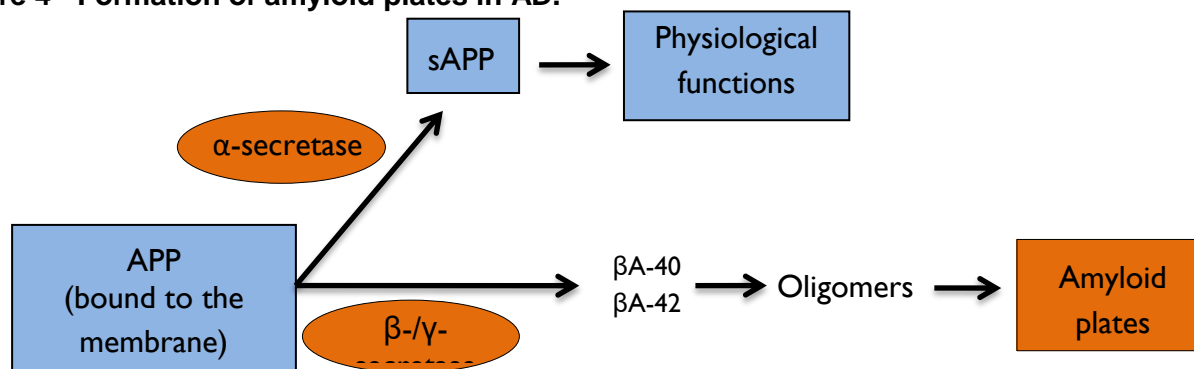
Legend: Adapted from RITTER, 2012.

β-CD, as well as its derivatives, methyl-β-CD (MβCD) and hydropropyl-β-CD (HPβCD), are capable of selectively extracting cholesterol from plasma membranes (BAR-ON et al., 2006).

Starting on the fact that several proteins may be internalised via endocytosis, a study whose purpose was to investigate the role of cholesterol in the caption of βA-42 in human cells was developed. MβCD was the selected cyclodextrin to act as cholesterol depletion agent (QINGHUA & SENFANG, 2006).

The results showed that the cell groups treated with MβCD had lower levels of cholesterol and internalised less quantity of the amyloidogenic protein. Thus, data indicate that cholesterol depletion affects endocytosis of βA-42 and therefore cell viability (QINGHUA & SENFANG, 2006).

In vitro studies have also shown that a reduction in the cholesterol levels by CDs leads to a lower activity of γ-secretase, leading to the reduction of the βA production, which propelled the performance of more tests (YAO et al., 2012).

A study performed in mice, that had a genetic mutation that lead to the abnormally high expression of APP, and to the consequence development of AD, assessed the modulator lipid activity of HP-β-CD. The results showed a significant reduction in cholesterol of the cell membrane and in the production of βA, allowing minimizing the brain area occupied by plates. The activation of genes involved in the transport and clearance of βA was also seen, which may have important consequences in the development of these molecules in the AD therapy (YAO et al., 2012).

The results presented in the above mentioned studies suggest that CDs may arise as potential therapeutic agents in the reduction of the progression and/or treatment of AD (QINGHUA & SENFANG, 2006; YAO et al., 2012).

5.1.2 Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disease with progressive loss of neurons of the dopaminergic system, which leads to the development of motor changes(40) and cognitive damage (BAR-ON et al., 2006).

This disease is associated with the accumulation of proteins in neurons, leading to the formation of intraneural inclusions called Lewis bodies (LB), which are directly related to neurodegeneration (ESPOSITO & CUZZOCREA, 2010). The main protein constituent of these inclusions is α-synuclein (α-syn), a soluble protein

with unfolded native form (RECCHIA et al., 2004). In normal physiological conditions, this remains located in the synapses, mainly in lipid rafts, where it can play a role in the regulation of neurotransmission (BAR-ON et al., 2006).

α-sin does not have a typical secondary structure, it is a very dynamic molecule, being the change of its structure very dependent of the environment around it. In pathological conditions, this can suffer conformational changes, deposit and aggregate (RECCHIA et al., 2004).

Performed studies showed that lipid environment promotes the transition of α-syn conformation, also acceleration its aggregation.(This fact suggests that the association of α-syn with lipids may be relevant in neurodegenerative disorders (RECCHIA et al., 2004; BAR-ON et al., 2006).

In PD, toxic forms of α-syn (incorrect fold) are commonly found associated with the membrane. Also it is possible that the lipid balance in the membrane is changed, resulting in an excessive accumulation of α-syn and in the formation of toxic oligomers in the membrane (BAR-ON et al., 2006).

As α-syn may interact with cholesterol in lipid rafts, the hypothesis of reducing agents of the cholesterol levels improve the pathological accumulation of it in PD was studied (BAR-ON et al., 2006).

A study performed assessed the effect of MβCD in the accumulation of α-syn in neural cells and in transgenic mice. In both, was seen that the extraction of cholesterol was accompanied by a reduction of the α-syn levels in the membrane and lipid rafts.

In transgenic mice, MβCD reduced the neural accumulation of α-syn and provided improvements of the degenerative changes, suggesting a possible therapy for PD.

The mechanism by which this decrease occurs is not completely clarified. However, it is thought that it is directly related to the ability to extract cholesterol from MβCD by complexation, which can then lead to redistribution of α-syn of the membrane aggregates into its soluble fraction.

In view of the obtained results, we can state that the use of compounds that reduce the cholesterol content in the lipid rafts may be seen as a potential tool in the treatment of synucleinopathies (BAR-ON et al., 2006).

CONCLUSION

CDs are surprising molecules, whose peculiar structure gives them unique properties that drive their application in several industries, namely pharmaceutical industry.

Their ability to form inclusion complexes allowed to overcome many of the obstacles found in the development of pharmaceutical formulations, being its importance as pharmaceutical excipient known and unquestionable.

Recently, several studies have indicated CDs as potential therapeutic agents, namely in targeting drugs and treatment of neurological diseases.

Current investigation has payed part of its attention in the specific delivery of drugs into the colon, resorting to CDs conjugates, because these can transport the unchanged drug until the end of the intestine.

Drugs administration as CDs conjugates did not reach higher therapeutic effects, however, it minimized the side effects associated with free drug. Thus, CDs revealed potential to be used in the treatment of local colon pathologies.

CDs conjugates also presented promising results in the treatment of oral cavity diseases and in local bone regeneration.

Studies performed allowed developing a specific drug delivery system, confounding the obstacles found in the treatment of oral cavity pathologies, and they also found, accidentally, the potential anabolic ability of CDs in bone regeneration.

In the area of neurological disorders, several studies associated high cholesterol levels with a higher level of neurodegeneration. CDs are targeted as study due to their known ability to extract cholesterol from plasma membranes.

Obtained results were promising, showing a lower formation of amyloid plates and lower accumulation of α-syn, in AD and PD, respectively.

More studies are needed, however, CDs urge expectations in order to appear as a new approach in reducing and/or treating these pathologies. Their potential as pharmaceutical excipient was already known, then it was already expected that they solved many problems associated with drugs delivery, however, it is now expected that in a near future CDs appear as therapeutic agents and fill many of the currently existing gaps.

FUTURE PERSPECTIVES

Since their discovery, new technologies based on CDs have continually been developed, revealing the huge potential of these molecules (VEIGA & FIGUEIRAS, 2011).

CDs multifunctional properties and bioadaptability still have the attention as specific

vectors in delivering drugs, with the common purpose of solving questions up to date without answer or with unsatisfactory answer (VEIGA, PECORELLI & RIBEIRO, 2006).

CDs found application in the development of new systems to deliver drugs by oral use, namely in the liposomes (VYAS, SARAF & SARAF, 2008), microspheres and nanoparticles, where they seek to resolve limitations presented by each one of these transport systems (VEIGA, PECORELLI & RIBEIRO, 2006). Among these limitations are solubility and stability problems, which when solved, allow the systems to include higher drug concentrations (VEIGA, PECORELLI & RIBEIRO, 2006; VYAS, SARAF & SARAF, 2008).

On the other hand, biotechnology progresses have allowed to increase the production of drugs based on proteins or peptides, however these have some characteristics that make their direct use difficult, namely chemical and enzyme instability, as well as weak absorption through biological membranes (CHALLA et al., 2005; VEIGA, PECORELLI & RIBEIRO, 2006). Interaction with CDs may end in changes of the chemical and biological properties of these drugs, appearing as a viable alternative to overcome some of the barriers found (VEIGA, PECORELLI & RIBEIRO, 2006). Also, CDs may promote the absorption of proteins and peptides, through solubility of lipids of biological membranes, with consequent increase of their permeability (VEIGA & FIGUEIRAS, 2011).

CDs have also been indicated as potential vectors for target sites, as the brain, colon or cancer cells (SALMASO & SONVICO, 2011). and also as potential vectors for genic therapy (CHALLA et al., 2005).

The development of systems to deliver drugs in cancer cells has been based on the formation of CDs conjugates with specific molecules, which have affinity for certain receptors or antigens present in the tumour, so the release of the drug, previously included in the CD cavity, occurs only on the surface of the target cells (VEIGA & FIGUEIRAS, 2011; SALMASO & SONVICO, 2011).

Thus, due to encapsulation of the drug and increase of the specificity of the release site, CDs may contribute to the increased treatment efficacy and to the decrease of side effects associated with chemotherapy (SALMASO & SONVICO, 2011). CDs are also studied in the development of non-viral vectors to deliver genes, in the expectation of being used as nucleic acid (NA) carriers. It is expected that these molecules improve permeability and stability, optimising therapeutic levels reached and minimizing the side effects inherent to the administration of NA (CHALLA et al., 2005).

The ability to extract cholesterol from the plasma membranes that the CDs have is also one of the characteristics responsible for their therapeutic application (DREYFUSS & OPPENHEIMER, 2011).

Despite their importance in neurological disorders having already been approached, CDs still appear as potential therapeutic agents in other pathologies of that area, namely in type C Niemann-Pick disease.

In summary, this is a neurodegenerative disease, characterised by an abnormal accumulation of cholesterol in the neurons. Studies performed showed that CDs administration, namely HPβCD, delayed the appearance of clinical symptoms and reduced the accumulation of cholesterol in the cells due to its ability of complexation and extraction from plasma membranes (PONTIKIS et al., 2013).

This CDs ability places them as potential therapeutic agents in pathologies as atherosclerosis and gives them unique abilities in preventing viral and bacterial infections (DREYFUSS & OPPENHEIMER, 2011).

CDs are exceptional molecules that continue to present new therapeutic applications and to open attractive pathways, making perceptible that they still have much unexploited potential in the pharmaceutical area.

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