REVIEW

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Use of prebiotics in oral delivery of bioactive compounds: a nanotechnology perspective

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The oral route is considered the most patient-convenient means of drug administration. In recent years there has been a tendency to employ smart carrier systems that enable controlled or timed release of a bioactive material, thereby providing a better dosing pattern and minimizing side effects. Nano-encapsulation systems (nanocarriers) offer important advantages over conventional drug delivery techniques. Nanocarriers can protect the drug from chemical/enzymatic degradation and enhance bioavailability. Prebiotics are ideal ingredients for the nano-encapsulation and oral drug delivery due to their natural ability to protect the encapsulated compound in the upper gasterointestinal (GI) tract. Here the potential of prebiotics for oral delivery of drugs and other bioactives is reviewed.

1. Introduction

The gastrointestinal tract is the preferred site of absorption for most therapeutic agents, due to several advantages such as ease of administration, patient compliance and cost. Oral delivery of bioactive compounds to the gastrointestinal (GI) tract can be employed for local or systemic administration. Compared to other routes of drug delivery, oral delivery leads to greater convenience, potentially less pain to the patient, and a reduced risk of cross infection, mostly associated with parenteral drug administration (Chen and Langer 1998; Florence and Jani 1993; Lopes et al. 2010; Sarasija and Hota 2000). However, physiological barriers such as the stomach environment and the enzyme activity of the GI tract are unfavourable for oral drug delivery. Secreted pancreatic enzymes in the intestinal lumen and membrane-bound brush-border enzymes may also cause a substantial loss of therapeutic activity. These have led to a great amount of research in the field of drug delivery from the standpoints of micro- and nano-encapsulation of the bioactive compounds. Prebiotics can be used as novel materials for the preparation of nanocarrier systems. In this entry, the role and advantages of prebiotics in oral bioactive delivery, using nanotechnology, is reviewed. The bioactive compounds covered here include, but are not limited to, therapeutic or diagnostic agents, vaccines, vitamins, hormones, nutraceuticals, food supplements and minerals.

2. Nanotechnology in bioactive delivery

Nanotechnology is a multidisciplinary scientific area, which employs a diverse array of tools and techniques derived from engineering, physics, chemistry and biology (Sahoo et al. 2007; Sahoo and Labhasetwar 2003). Advancements in nanoscience and nanotechnology have made it possible to manufacture and characterize sub-micron bioactive carriers on a routine basis. The delivery of bioactives to target sites inside the body and their release behavior is directly affected by particle size. Compared to micrometer-sized carriers, nanocarriers provide more surface area and have the potential to increase solubility, enhance bioavailability, improve controlled release and enable precision targeting of the entrapped material to a greater extent (Mozafari 2006). As a result of improved stability and the possibility for targeting, the amount of material required for a specific effect when encapsulated in, or incorporated to, a nanocarrier is much less than the amount of the same material required when unencapsulated. Bioactive targeting to its required site of action is essential to eliminate adverse effects. A timely and targeted release improves the effectiveness of bioactive compounds and ensures optimal dosage, thereby improving cost-effectiveness of the formulation. In general, reactive or sensitive bioactive material can be turned into stable ingredients through encapsulation by a nanocarrier system (Khosravi-Darani et al. 2007; Mozafari 2006).

Many of the current nanocarrier systems are in fact conventional drug delivery systems that happen to be in the nanometer size range. These include nanoliposomes, nanoparticles, dendrimers, polymeric micelles, niosomes and nanocrystals (Mozafari et al. 2008; Park 2007). In addition to reducing the frequency of drug administration, and thus improving patient comfort, novel delivery systems offer protection and improve the pharmacokinetics of easily degradable compounds, such as polypeptides and polynucleotides, which often have short half-lives *in vivo* (Orive et al. 2003). For the pharmaceutical industry, the field of bioactive delivery represents a strategic tool for expanding drug markets, because new delivery technologies could

reformulate/repackage classical drugs, offering a competitive edge after the upcoming patent expirations and avoiding competition from generics.

Nanocarrier-mediated drug delivery has made it possible to target chemotherapy agents directly to tumors, hence reducing side effects (Hughes 2005). In fact, nanocarrier technology is the foundation upon which most nanotechnological cancer therapies are based. In an *in vivo* study, Lamprecht et al. (2005) employed tacrolimus (FK506) loaded poly(lactic-co-glycolic acid) nanoparticles entrapped in pH-sensitive microspheres. The FK506 nanoparticles were administered orally or rectally to rats suffering from pre-existing experimental colitis. Results showed successful incorporation of FK506 nanoparticles and release of the drug as well as the nanoparticle into the tumor environment as opposed to the surrounding tissue. In an *in vitro* study, Morgan et al. (2006) showed that encapsulation of camptothecin drugs in dendrimers increased their solubility, cellular uptake, and cellular retention and consequently improved the anticancer activity of the drug. Furthermore, it was shown that nanoparticle formulations of irinotecan possess better efficacy, compared with the free (un-encapsulated) form of the drug (Williams et al. 2003).

3. Prebiotics

Prebiotics can be defined as: "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a number of bacteria in the colon that can improve host health" (Gibson and Roberfroid 1995). Emphasis on the colon in this definition may be too restrictive as many monogastric animals and humans support a considerable degree of bacterial fermentation in the upper GI tract (Gaskins 2001; Wilson 1997). Furthermore, in recent years focus has shifted towards the concept of the need for a stable and complex commensal bacterial community as a pre-requisite for a healthy gut ecosystem (Konstantinov et al. 2004; Verstegen and Williams 2002). Such considerations have led to further elaboration of the definition of a prebiotic as: "a non digestible dietary ingredient that beneficially affects the host by stimulating the activity, in terms of fermentation end products, and stability of the diverse commensal microbiota in different parts of the gastrointestinal tract, depending on the fermentability of the dietary ingredient itself" (Awati 2005; Awati and Moughan 2006). Prebiotics include oligosaccharides of fructose and galactose as well as lactulose, which are non-digestible by human digestive enzymes but can be metabolized by colonic bacteria to produce short-chain fatty acids such as butyrate (Fig. 1). Other prebiotic materials include lactosucrose, soybean oligosaccharides, isomalto-oligosaccharides, xylo-oligosaccharides and gluco-oligosaccharides. Some of the naturally occurring as well as synthetic polysaccharides have been studied extensively for their prebiotic properties in humans as well as in animals. These include inulin, guar gum, resistant starch, pectins, chitosan and lactulose (Awati et al. 2005; Kelly 2008). Chemical structure of some prebiotic molecules are depicted in Fig. 2.

From a physiological point of view the main role of a prebiotic is to provide a preferred substrate for the potentially beneficial colonic bacteria. Most of the compounds investigated for such prebiotic properties are dietary fibers. Generally, soluble fibers are better energy substrates for gastrointestinal microorganisms than are insoluble fibers (Fahey et al. 2004). Ingestion of prebiotics is followed by an increased excretion of breath-hydrogen, as reviewed by Cummings et al. (2001). This is a proof that the prebiotic fiber components are generally well fermented in the GI tract, although the rate of fermentation may vary depending on the source of the fiber component and the host species.

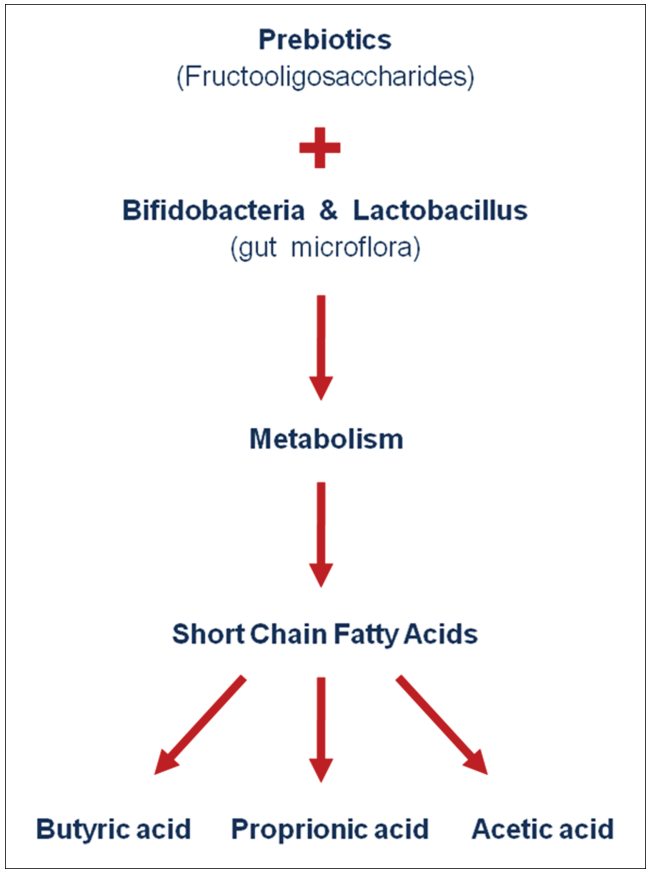

Fig. 1: Prebiotic metabolism by the microflora of the gastrointestinal tract

The important characteristics of prebiotics, their role as functional food ingredients and their contribution to general health benefits are well documented in the literature (Andrieux 2001; Bengmark 2002; Berg 1998; Blaut 2002; Chow 2002; Collins and Gibson 1999; Fooks et al. 1999; Marfarlane and Cummings 1999; Ogueke et al. 2010; Rastall and Maitin 2002; Roberfroid 2002). However, utilization of prebiotics as a preferred substrate by commensal microbiota for site-specific bioactive delivery is a relatively new, but highly promising, field of research and development.

4. Fermentability of prebiotics

Detailed knowledge on the fermentability of different prebiotic substances, which can be employed as suitable candidates for bioactive encapsulation, is necessary. Information on the possible site of degradation of the particular prebiotic substance would add to the accuracy of the prediction of bioactive delivery using the prebiotic approach. Bifidobacteria have been identified as preferred target microorganisms for prebiotic compounds (Fig. 1) (Vernazza et al. 2005). Experimental studies using human gut bacteria have shown that galactooligosaccharides and fructooligosaccharides are fermented by those bacteria with a resultant decrease in pH of the medium during anaerobic fermentation (Gibson and Wang 1994; Wang and Gibson 1993). Several *in vitro* techniques for determining rate and degree of fermentability have been suggested. Coles et al. (2005) have described some of the most common techniques used for the study of fermentability of different dietary fermentable fibers. Among the different available procedures the *in vitro* gas production technique has been recently used to demonstrate differences in the fermentability of different carbohydrate substrates (Williams et al. 2005). Using the *in vitro* gas production

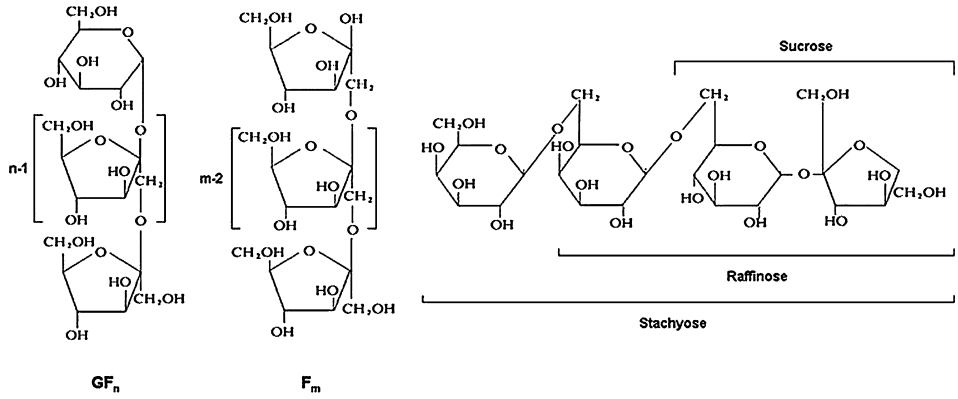

Fig. 2: Chemical structure of fructooligosaccharides (A) and galactooligosaccharides (B). G: glucos; F: fructose; n and m indicate total number of fructose moieties in each molecule

technique, Awati et al. (2005) have shown that different fermentable carbohydrates are fermented in different parts of the GI tract of weanling piglets. The use of *in vitro* fermentation techniques coupled with obtaining inocula from specific sites of the GI tract to measure fermentability of candidate substrates will lead to more accurate site-specific delivery of the bioactive compounds following oral administration (Awati 2005; Awati and Moughan 2006).

5. Use of prebiotics in oral delivery

Within contemporary pharmaceutical sciences different routes of administration have been developed for efficient bioactive delivery. Still, oral administration remains the most convenient method of bioactive delivery with highest compliance rates among patients. One of the main challenges in bioactive delivery is finding a means of carrying the bioactive in a stable form to the target site (Fahmy et al. 2005; McConnell et al. 2009; Udagawa and Wood 2010). It is important that the particular carrier system protects the bioactive compound from immunological as well as physiological processes, such as uptake by the reticuloendothelial system (RES, in case of parenteral applications), or digestion (in case of oral delivery), until it reaches the desired target site.

After oral administration of a conventional dosage form it dissolves or erodes within the GI tract and the bioactive is absorbed across GI epithelia (Perera et al. 2010). The GI tract is inhabited by more than 400 bacterial species, each having a specific location in the tract. The distal part of the intestine (i.e. colon) is inhabited by a large variety of gram negative microflora. This flora produces a vast number of enzymes that are being used for the formulation of colon-specific bioactive delivery systems (Sinha and Kumaria 2003). A number of microbially activated systems for colon-specific bioactive delivery are under investigation. These include prodrugs and synthetic or naturally occurring polymer-based carrier systems (Sinha and Kumria 2004). Several methods have been developed for oral delivery of bioactive material. One of the oldest and the most commonly employed method uses enteric polymers as coating materials over tablets, granules, or pellets. These rely upon the difference in pH values in the GI tract (Rubinstein 1995). Others include time-controlled release systems (Niwa et al. 1995), pressure controlled release systems (Muraoka et al. 1998), prodrugs (Sinha and Kumria 2004), polysaccharide-based delivery systems (Sinha and Kumaria, 2001) and osmotically controlled release systems (Theeuwes et al. 1993). Using prebiotics for nanoencapsulation of bioactives is based on the fact that prebiotic material can be used to coat a bioactive compound, thus allowing safe passage through the stomach and upper digestive tract without release. Prebiotics have the useful property that they are indigestible substrates (resistant to mammalian gut enzymes), can easily pass the gastric barriers and be fermented by microbiota to release their load (Awati and Moughan 2006). During the last decade several authors have reported the successful use of dietary fiber components for the encapsulation of different bioactive compounds (Bassett and Cash 2008; Fung et al. 2010; Singh and Chauhan 2009, 2010). The idea of using the prebiotic substances specifically for colonic drug delivery in humans has been reviewed by Sinha and Kumaria (2003) and Das et al. (2010). The authors discuss three different systems that have been developed and studied for oral bioactive delivery based on exploiting the microbial population of the GI tract, as explained below.

5.1. Prodrugs

A prodrug can be defined as a pharmacologically inactive derivative of a parent drug that requires spontaneous or enzymatic transformation *in vivo* to release the active drug (Ferriz and Vinsova 2010; Jung and Kim 2010). For oral drug delivery, prodrugs are designed to undergo minimal absorption and hydrolysis in the upper GI tract but to undergo enzymatic hydrolysis in the colon, thereby releasing the bioactive moiety from the carrier. Despite high level of site specificity, Sinha and Kumaria (2003) admonish that the prodrug approach is not very versatile as the ability to form a prodrug depends upon the functional groups available on the drug moiety for chemical linkage. Furthermore, prodrugs are considered new chemical compounds and thus need extensive characterisation and regulatory approval before being used as drug carriers (Awati and Moughan 2006; Gupta et al. 2009).

5.2. Azo polymeric prodrugs/azo polymeric coating

Bioactive compounds coated with biodegradable polymers can be degraded in the GI tract due to the influence of colonic microorganisms. Both synthetic as well as naturally occurring polymers are used as bioactive carriers for oral delivery of the bioactive compounds. Synthetic polymers have been used to form polymeric prodrugs with azo-linkages between the polymer and the bioactive moiety (Awati and Moughan 2006). These biodegradable polymers, especially azo polymers, have been considered for targeting an orally administered drug to the colon. Upon passage of the dosage form through the GI tract, it remains intact in the stomach and small intestine where very little microbial degradation activity is present. Release of the bioactives

from azo polymer-coated formulation is believed to take place after reduction and thus degradation of the azo bonds by the azo reductase enzymes released by the azo bacters present in the colonic microflora (Das et al. 2010). The metabolism of azo compounds by intestinal bacteria has been studied extensively and numerous polymers have been evaluated to meet this end. However, these polymers have similar disadvantages as was the case for prodrugs. Polymeric prodrugs, being new chemical entities, require a comprehensive toxicological assessment before being used as bioactive delivery systems (Hong et al. 2005; Shantha et al. 1995).

5.3. Polysaccharide-based delivery systems

Use of naturally occurring polysaccharides is a promising approach for oral bioactive delivery. These compounds are inexpensive, found in abundance, and are available in a variety of natural resources and with varied properties (Hovgaard and Brondsted 1996; Jain and Jain 2008). Many of the polysaccharide-based carrier systems are resistant to digestion in the upper GI tract and when they arrive in the colon the glycosidic linkages are hydrolysed by colonic microbiota to release the drug. Several different polysaccharides, which have been used in drug delivery systems, are reviewed by Sinha and Kumaria (2003). These polysaccharides mainly include naturally occurring polysaccharides obtained from plants (amylose, guar gum, inulin), animals (chitosan, chondroitin sulphate), algae (alginates) or microbes (dextran) (Chayed and Winnik 2007). It has been shown that the bioactive release from amorphous amylose-coated products was accelerated in the fermentative environment of the colon. This was attributed to the bacterial digestion of the amylose component of the film coat producing pores for drug diffusion (Wilson and Basit 2005).

In humans, considering the lower amount of microbial activity in the small intestine, application of prebiotic approach to bioactive delivery is mainly restricted to the large intestine. However, in specific disease conditions such as small bowel bacterial overgrowth, in which the main consequence of small bowel dysmotility is overgrowth of the microbial community, microbial activity in the small intestine increases (Husebye 1995). In such a situation, oral administration of an antimicrobial agent coated with fast fermenting indigestible soluble fiber, may improve the efficacy of the antimicrobial drug by site specific delivery. This is a 'Trojan horse' approach to combat pathogens. The bacteria degrade the prebiotic carrier system containing the antimicrobial drug, leading to their own self-destruction (Awati and Moughan 2006; Gupta et al. 2009).

There are several other strategies for oral bioactive delivery among which liposomes (Colas et al. 2007; Mozafari 2005; Takeuchi and Sugihara 2010) and some other lipid-based carrier systems (e.g. archaeosomes, virosomes, transferosomes) (Gasco et al. 2009; Mortazavi et al. 2005; Mozafari et al. 2005) are the most studied and widely applied technologies. As discussed by Mozafari and colleagues, these types of bioactive delivery systems may have problems due to toxicities related to product manufacture procedures (Mortazavi et al. 2007a,b). Moreover, due to low pH and the presence of digestive enzymes (e.g. lipases) these lipidic carriers cannot generally pass to the lower GI tract in intact form. With respect of these issues, prebiotics being natural dietary ingredients hold a distinctive advantage both in terms of safety and efficacy. In addition, in veterinary therapeutics using fermentable substrates with variable fermentabilities as coating material will be an advantage. Different animal species have diverse active microbial communities in different parts of their GI tract other than the colon. For instance, chickens have a very well developed microbial community in the crop, and in the caecum, whereas the pig has active microbial fermentation in the stomach, ileum and the large intestine (Awati and Moughan 2006). Consequently, prebiotic-based bioactive delivery systems can be used both in human as well as in veterinary medicine to target their load to different parts of the GI tract.

6. Conclusions

The gastrointestinal tract has been the most popular route for bioactive delivery despite some known disadvantages. Furthermore, the current trend in oral bioactive administration is to explore possibilities for targeted delivery within the GI tract. Towards this end, utilisation of prebiotic compounds for the nano-encapsulation and delivery of bioactive material holds a promising future. The prebiotic-based nanocarrier formulations can improve the bioavailability of bioactive compounds that are prone to be degraded along the upper GI tract. This is a multifunctional approach to bioactive delivery considering the health benefits of prebiotic consumption and the fact that prebiotics are preferred substrates for microbiota, ensuring safe and site-specific delivery of the bioactive material. Exploitation of microbial activity in terms of bioactive targeting, however, will need to rely on an extensive knowledge of the fermentability of the prebiotic substrates and the composition and activity of the microbiota at specific sites of the GI tract. These characteristics, in turn, will vary depending on the host animal species.

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