Microbially triggered drug delivery to the colon

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Received 22 March 2002; received in revised form 4 October 2002; accepted 11 October 2002

Abstract

Increasing acceptance of protein- and peptide-based drugs necessitates an investigation into the suitability of various sites for their administration. Colon is being investigated for delivery of such molecules. Colon-specific drug delivery is designed to target drug molecules specifically to this area. Development of site-specific delivery systems may exploit a specific property of the target site for drug activation/release. The gastrointestinal tract is inhabited by over 400 bacterial species, each having a specific niche in the tract. Colon, the distal part of the intestine is inhabited by a large variety of gram negative microflora. This flora produces a vast number of enzymes which are being exploited for formulation of colon-specific drug delivery systems. A number of microbially activated systems for colon-specific drug delivery are being evaluated. These include prodrugs and synthetic or natural polymer-based delivery systems. This article aims at reviewing the various microbially activated drug delivery systems for colon-specific drug delivery with specific reference to the microflora of the various segments of the gastrointestinal tract and their role in targeting drug delivery to the colon.

Keywords: Microflora; Colon-specific drug delivery; Prodrugs; Polysaccharides; Colon targeting

1. Introduction

Generally the small intestine is considered as the primary site for drug absorption and therefore the preferred part of the gastrointestinal tract (GIT) for targeting with various controlled release technologies. Increasingly, interest has developed in directing drugs and dosage forms to effect primary drug release to the colon. The various categories of drugs being targeted to this site include drugs, which are unstable/unabsorbed in the upper GIT, and drugs, which are required for treatment of local colonic pathologies. Additionally, this site may be useful for delivery of those drugs where a delay in drug absorption is required from a therapeutic point of view e.g. in case of nocturnal asthma, angina etc. (Kinget et al., 1998; Rubinstein, 1995).

Colon, as a site offers distinct advantages on account of a near neutral pH, a much longer transit time, reduced digestive enzymatic activity and a much greater responsiveness to absorption enhancers. This enables the visualization of this distal part of the GIT as a site for delivery of various drug molecules including proteins and peptides.

For local pathologies of the colon, colon specific drug delivery, not only increase the bioavailability of the drug at the target site, reduce the dose to be administered but also would reduce the side effects.

Various systems have been developed for colon specific drug delivery. These include, systems developed using pH-sensitive polymers (enteric coating polymers), time dependent release systems or enzymatically-controlled delivery systems. Enteric-coated systems are most commonly used for colonic drug delivery and constitute majority of commercially available preparations for colon targeting (Leopold, 1999). However, a disadvantage of these systems is that a substantial amount of drug may be released in the small intestine before the delivery system arrives in the colon. Further the pH-difference between the small intestine and the large intestine not being very pronounced, these delivery systems do not allow reproducible drug release (Leopold, 2000). Limitation of time dependent release systems is that they are not able to sense any variation in the upper GIT transit time. Since most of the time dependent release systems are enteric coated, they can overcome the large variation in the transit time of stomach. The in vivo variations in small intestinal transit time however, may lead to drug release in the small intestine or the terminal colon.

Apparently, a more expedient approach for site-specific
drug delivery to the colon is by developing systems, which can sense arrival into the colon and release the drug upon activation. Such systems can be formulated utilizing some specific property of the colon in comparison to the other parts of the GIT. The gastrointestinal tract is inhabited by a variant microflora all along. The flora becomes diverse and luxuriant in the colon (Fig. 1). It is the presence of these microflora in the colon, which is being utilized for site-specific delivery. This review aims at reviewing the various microbially activated drug delivery systems developed for colon specific drug delivery. Special emphasis has been paid to the variation in the microflora present in the various regions of the GIT and exploitation of this variance for colonic drug delivery.

2. Gastrointestinal ecosystem

All ecosystems contain habitats and niches for microbes. Each habitat contains niches that are occupied normally by indigenous microbes called the autochthonous microbes. However, non-indigenous microbes may also be found in a habitat. These microbes may just be passing through the habitat or they may fill in a niche, which has been vacated due to some reason by the autochthonous inhabitants. These are known as allochthonous microbes. However, once the system comes to normal, the indigenous microbes reoccupy the habitat (Savage, 1977a).

Gastrointestinal ecosystems are open, integrated, interactive units containing 400–500 microbial inhabitant species forming the main reservoir of bacteria in the human body. It has a total live population of $10^{13}$ eukaryotic cells that make up the human body (Savage, 1977a). In a normal adult, a microbial community, consisting of many indigenous microbes, colonizes each of these habitats. Each one of these species occupies a niche in the habitat and contributes to the economy of the whole system thus, forming an enormously complex ecosystem, which includes both aerobic and anaerobic microorganisms. The aerobes are outnumbered by the anaerobes by a factor of 1000. At any point of time some non-indigenous microbes from food, water, another habitat in the GIT, soil, air, skin, mouth, respiratory tract, etc. may also be present in these tracts.

The gastrointestinal tract starts from the oral cavity, into the esophagus, stomach, small intestine and finally the large intestine. It has been so designed that normally, despite our eating of any type of food, our natural defense mechanisms take care of the usual contaminants and microbes found in the food. Further as we eat food, drink or talk many microbes enter into our oral cavity. With the help of saliva, they pass through the esophagus into the stomach. The acidic pH of the stomach and the intestinal peristaltic waves kill most of the microbes and a very low number of microbes pass further (Macy, 1979; Simon and Gorbach, 1984). Small intestine is that part of the GIT, where the main processes of digestion and absorption take place. Had the stomach not lowered the microbial concentration, the microbes would have competed with the normal small intestinal epithelial cells for the products of digestion, resulting in decreased nutrient availability to the host system. Latter to the ileocecal value i.e. in the large intestine, the flora present is luxuriant, containing a wide variety of different bacteria. This part of the GIT functions.
mainly as a site for reabsorption of water and residual carbohydrate fermentation.

The importance of the intestinal microflora and more specifically its composition in the physiological and pathophysiological processes in the human gastrointestinal tract is becoming more and more evident (Salmiren et al., 1995; Kasper, 1998). Recognizing the importance of these microorganisms, the use of prebiotics and probiotics has gained importance (Collins and Gibson, 1999; Majamaa and Isolauri, 1997).

2.1. The Stomach

The stomach is the most dilated part of the alimentary canal and is situated between the esophagus and the small intestine. Its shape and position are modified by changes within itself and by the surrounding viscera. It has a capacity of 1500 ml in a normal adult (Bannister, 1995). Microorganisms from the mouth and saliva enter into the stomach through the esophagus. Saliva has around $10^7$ CFU ml$^{-1}$ of bacteria. The number of aerobic and anaerobic bacteria is nearly the same. As these bacteria enter the stomach, due to acidic pH in this region, resulting from secretion of hydrochloric acid by the parietal cells, most of these are destroyed (Hungate, 1978). When food is present the gastric pH rises to more than 4 and the bacteria proliferate to about $10^8$–$10^9$ CFU ml$^{-1}$. But once the food mixes with the gastric juice, the pH falls down and only the acid-resistant bacteria survive (Drasar et al., 1969; Barbara and Friend, 1992). The microflora of the stomach is normally sparse and bacterial concentration is less than $10^3$ CFU ml$^{-1}$. Only those microorganisms are able to inhabit the stomach, which are sufficiently acidic to grow at such a high hydrogen ion concentration (Shifrine and Pfaff, 1958; Barbara and Friend, 1992). Additionally, only those microorganisms, which are able to associate with the epithelial surfaces, can exist in this region. This is a critical characteristic for maintenance of microbial community in the stomach since here the rate of passage of the contents exceeds the rate of multiplication of microbes. The microflora here is predominantly gram-positive and aerobic. The most commonly isolated species are Streptococci, Staphylococci, Lactobacilli and various fungi (Gorbach et al., 1967a; Stanier et al., 1976). Oral anaerobes such as Peptostreptococcus, Fusobacterium may be present in low numbers but Coliforms, Clostridium are distinctly uncommon.

2.2. The small intestine

The small intestine is a coiled tube extending from pylorus to the ileocecal value, where it joins the large intestine. It is 6–7 m long with gradually diminishing diameter towards its terminal end. It is divided into three parts, duodenum, jejunum and ileum. The first short sessile section is the duodenum, followed by a long, greatly coiled part forming the two-fifth portion, the jejunum. The distal three-fifth part forms the ileum (Bannister, 1995). The microflora of the duodenum resembles that of the stomach whereas, that of the ileum resembles the luxuriant microflora of the colon. The bacterial concentration in the duodenum is of the order of $10^3$–$10^4$ CFU ml$^{-1}$ and the predominant species are aerobic and gram positive. These include Streptococci, Staphylococci, Lactobacilli and anaerobic Veillonella. The bacterial growth is reduced due to chemical factor (e.g. bile juice, lysozyme) and peristalsis. Coliforms and other anaerobic bacteria may be found in low concentration. In the jejunum and the upper ileum very few microorganisms are present which include Lactobacilli and Enterococci. In the distal ileum, gram-negative bacteria begin to outnumber the gram-positive organism. The bacterial concentration becomes high. Coliforms are consistently present and anaerobic bacteria such as Bacteroides, Bifidobacterium, Fusobacterium, Clostridium are found in high concentration (Gorbach et al., 1967a; Gorbach and Levin, 1970; Drasar et al., 1969; Drasar and Shiner, 1969; Gorbach, 1971; Thadepalli et al., 1979). Also, Sterptococci, (Sterptococcus faecalis), Staphylococci, Lactobacilli, Clostridium perfringes, Veillonella and at time Escherichia coli may be found.

2.3. The large intestine

The large intestine extends from the distal end of the ileum to the anus. Human large intestine is about 1.5 m long. Its calibre is higher near the cecum and gradually diminishes to rectum, where as it enlarges just above the anal canal. The cecum forms the first part of the large intestine and leads to the right colon or the ascending colon followed by the transverse colon, the descending colon, sigmoidal colon, rectum and the anal canal.

Distal to the ileocecal sphincter, the bacterial concentration increases sharply and the colon has a microflora of $10^{11}$–$10^{12}$ CFU ml$^{-1}$ and approximately one third of the fecal dry weight consists of bacteria (Moore and Holland, 1975; Savage, 1977; Simon and Gorbach, 1984) (Fig. 1). This increase in bacterial flora in this region can be attributed to a near neutral pH caused by neutralization of the contents of the bowel by the intestinal juice and also due to the lowered speed of the contents in the large intestine. Since this part of the GIT mainly functions as a site for reabsorption of water from the chyme, no much mixing and propulsive movements are required and the colon mainly functions as a reservoir. This slow passage of contents in this segment leads to stagnation of flowing stream. The rate of passage of luminal contents does not exceed the doubling time of the bacteria and they proliferate. The main bacterial population present is that of oxygen-intolerant anaerobic bacteria of various types. The anaerobic bacteria outnumber the aerobes by a factor of $10^2$–$10^4$. As many as 400 different bacterial species are found. The predominant species isolated include Bac-
The gut flora has also been known to have a considerable relationship where, the host derives many benefits. The main products of digestion of carbohydrates and proteins in the colon are short chain fatty acids (SCFA) (Gibson et al., 1995; Cummings and Macfarlane, 1991), which are rapidly absorbed from the large intestine and stimulate sodium and water absorption (Ruppin et al., 1980; Roediger and Moore, 1981) and thereby, contribute to salt and water homeostasis in the colon. These fatty acids are used as fuels by the epithelium (Roediger, 1980, 1982; Von Engelhardt and Rechkemmer, 1983). Butyrate regulates nucleic acid metabolism of the epithelial cells and also helps in maintaining the health of the epithelium (Cummings and Englyst, 1987). The resident flora resists colonization of exogenous pathogenic bacteria in addition to restricting the normal flora in the tract (Gomez et al., 1999; Iglewski and Gerhardt, 1978). Intestinal bacteria have been found to have a role in synthesis of vitamin B and K and in stimulation of the immune system of the gut. The gut flora has also been known to have a considerable role in the metabolism of foreign compounds, e.g. in metabolism of sulphasalazine, isonicotinuric and salicyluric acids, 1-dopa, lactulose, digoxin, cyclamates, etc. (Goldman, 1978; Boxenbaum et al., 1979).

2.4. Variation in the normal flora

The bacterial population of the GIT may considerably vary from one animal species to another and even from one individual to another, however, the enteric flora in a given individual remains remarkably stable. Though, the environment in which the host exists influences his enteric flora, a striking effect on the flora is not observed. Extreme unphysiologic alteration in diet, influences the kind and number of microorganisms that populate the intestinal canal (Donaldson, 1968; Gorbach et al., 1967b), though this has only slight effect on the colonic flora.

Alteration in composition of the GIT flora occurs under certain diseased states, e.g. in acute diarrhoea, the resident flora may be eclipsed by a pathogen; anatomic physiologic derangement of GIT may lead to bacterial overgrowths which in turn may cause variation in microflora; in cholera a very high concentration of Vibrio cholera in the small intestine is found with a marked reduction in anaerobic population of the large bowel; in tropical spruce, the aerobic microorganism outnumber the anaerobes; etc. Keighley et al. (1978) investigated the influence of inflammatory bowel disease (IBD) on the intestinal microflora. Profound changes in microflora of the small and large intestine were found in some patients with Crohn’s disease but these were not observed in patients with ulcerative colitis.

Under normal physiological states, the microflora of the digestive tract is a complex microbial ecosystem, which is well balanced and undergoes specific changes in ratio of aerobic and anaerobic microorganism, in an aboral direction. It typically has an absence of anaerobic microorganisms in the stomach, which conversely are in absolute predominance in the colon.

3. Colon-specific drug delivery: role of microflora

The vast microflora in the colon fulfills its energy needs by fermenting the various types of substrates that have been left undigested in the small intestine. Substrates, like glucose, which can be easily utilized by the microorganisms hardly, reach this part of the intestine since glucose and other easily digestible substrates are well absorbed or utilized in the upper GIT. The indigestible portion of the food, i.e. truly the physiological roughage (McBee, 1970) including di-, tri-polysaccharides, mucopolysaccharides, etc. (Rubinstein, 1990) reach the colon. To utilize this roughage as a source of carbon, bacteria produce a wide range of reductive and hydrolytic enzymes. These include β-glucuronidase, β-xylosidase, α-arabinosidase, β-galactosidase, nitroreductase, azoreductase, deaminase, urea hydroxylase etc. (Scheline, 1973; Kinget et al., 1998).

With the knowledge that the anaerobic bacteria of the colon are able to react to the constantly changing mixture of complex carbohydrates entering the colon by recognizing a variety of substrates and producing the appropriate digestive enzyme (Salyers et al., 1978), various systems have been developed for drug delivery to this part of the GIT. These include prodrugs (Sinha and Kumria, 2001a) and systems based upon biodegradable polymers which are specifically degraded by these enzymes (Van den Mooter et al., 1995; Sinha and Kumria, 2001b).

Also the presence of the vast microflora causes changes in redox potential, which are an expression of total metabolic and bacterial activity. The redox potential of the proximal small bowel has been found to be $-67±90$ in the distal small bowel; it is $-196±97$ and $-415±72$ in the right colon (Stirrup et al., 1990; Wilding et al., 1994). Redox mediators, such as benzyl viologen and flavin mononucleotide, act as shuttles between the intracellular enzymes and the extracellular substrates (Grim and Kopecek, 1991). These microflora-induced changes in the redox potential have been used as a highly specific mechanism for targeting drugs to the large bowel. This causes reduction of bonds like the azo bonds and disulphide bonds. Various drugs are being linked to such
carrier moieties with these bonds. These linkages are increasingly being exploited for drug delivery to this part of the GIT. A number of drug-carriers for colon specific drug delivery have been designed which either utilize the presence of enzymes or the redox potential of this particular part of the GIT for drug targeting. These have been discussed briefly in the next part on prodrugs.

3.1. Prodrugs

Prodrug is pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation in vivo to release the active drug. For colonic delivery of drugs, prodrugs are designed to undergo minimal absorption and hydrolysis in the tracts of the upper GIT and undergo enzymatic hydrolysis in the colon, there by releasing the active drug moiety from the carrier.

The metabolism of azo compounds by the intestinal bacteria is one of the most extensively studied bacterial metabolic processes. Both intracellular and extracellular reduction has been observed. Back in 1942 it was realized that sulphasalazine given for the treatment of rheumatoid arthritis was also useful in patients with inflammatory bowel disease (IBD). Furthermore, Khan et al., 1977 found that the active moiety effective in IBD was 5-aminosalicylic acid (5-ASA) and sulphapyridine (SP) only acted as a carrier. The azo bond between these two moieties undergoes reduction in the colon. However, due to a number of side effects associated with SP studies were conducted to find a suitable carrier, which could facilitate delivery of 5-ASA to the large intestine with minimal side effects. This led to formation of ipsalazide, balsalazide (Chan et al., 1983) and finally olsalazide, where two molecules of 5-ASA were joined together and one acted as a carrier for the other (Willoughby et al., 1982) (Table 1).

A number of other linkages susceptible to bacterial hydrolysis specifically in the colon have been prepared where the drug is attached to hydrophilic moieties like amino acid, glucuronic acid, glucose, galactose, cellulose, etc (Table 1). Amino acids consisting of polar groups like –NH₂ and –COOH have been used as carriers for colon specific delivery of drugs. Nakamura et al. (1992a,b,c) prepared a series of prodrugs for colon drug delivery using these amino acids. These prodrugs were designed to be bulky and hydrophilic to remain unabsorbed in the upper GIT. However, the intestinal microflora of the colon hydrolyzed the drug–amino acid linkage and the drug was released free in the lumen of the colon. A number of other prodrugs prepared using these amino acids have also been outlined in Table 1.

Sugar moieties like glucose, galactose and cellobiose have also been conjugated to drug moieties to form glycosides. These glycosides have been found to be highly site specific for selective quantitative drug delivery to the colon. The drug–sugar glycosidic linkage was found to be selectively hydrolyzed by glucosidase, galactosidase or cellobiosidase enzymes of bacterial origin in the cecum and colon (Friend and Chang, 1985).

Another type of conjugates which have also been found to be quite site specific are the glucuronide conjugates where glucuronic acid is conjugated to the drug moieties. These conjugates were stable in tracts of upper GIT and the presence of glucuronidase in the tracts of colon, hydrolyze the drug–glucuronic acid linkage releasing the drug free in the colon (Table1).

The high site specificity of prodrugs clearly indicates the involvement of the colon for the prodrug to drug conversion. The role of the bacterial flora in carrying out this conversion is strengthened by the studies carried out on germ free animals or animals pretreated with antibiotics like kanamycin. These studies have shown that the hydrolysis of the prodrug to active drug moiety was significantly inhibited under such conditions (Nakamura et al., 1992d; Haeberlin et al., 1993).

Prodrug approach is not very versatile approach as its formation depends upon the functional groups available on the drug moiety for chemical linkage. Furthermore, prodrugs are new chemical entities and need a lot of evaluation before being used as drug carriers.

3.2. Azo polymeric prodrugs/azo polymeric coating

Newer approaches are aimed at use of polymers as drug carriers for drug delivery to the colon. Both synthetic as well as naturally occurring polymers are used for this purpose. Some synthetic polymers have been used to form polymeric prodrug with azo-linkage between the polymer and drug moiety. These have been evaluated for colon specific drug delivery. One such prodrug consisting of a non-absorbable sulphanilamido ethylene polymer linked to 5-ASA has been found to be more effective than sulphasalazine in reducing inflammation in guinea pig (Brown et al., 1983) (Table 1).

Various azo polymers have also been evaluated as coating materials over drug cores. These have been found to be similarly susceptible to cleavage by the azoreductase in the large bowel. Coating of peptide capsules with polymers cross-linked with azaaromatic group have been found to protect drug from digestion in the stomach and small intestine. In the colon, the azo bonds are reduced and the drug is released (Saffran et al., 1986, 1988). Also azo polymers have been found to deliver vasopressin and insulin to the systemic circulation after the oral administration. Few other azo-bond containing polymers evaluated as conjugates or as coating materials have been outlined in Table 2.

Though a number of these synthetic azo polymers have been evaluated for the above purpose, these being new chemical entities require a detailed toxicological study to be performed before being used as drug delivery systems. Considering this, use of naturally occurring simple sac-
### Table 1
Prodrugs evaluated for colon-specific drug delivery with their in vitro/in vivo performance

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Drug investigated</th>
<th>Linkage hydrolysed</th>
<th>In vitro/in vivo model used</th>
<th>Performance of the Reference</th>
<th>Reference</th>
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<tr>
<td>Azo conjugates</td>
<td></td>
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</tr>
<tr>
<td>Sulphapyridine (SP)</td>
<td>5-ASA</td>
<td>Azo-linkage</td>
<td>Human</td>
<td>Site specific with a lot of side effects associated with SP</td>
<td>Khan et al., 1977</td>
</tr>
<tr>
<td>p-Aminobiphenurate</td>
<td>5-ASA</td>
<td>Azo-linkage</td>
<td>Human</td>
<td>The prodrug was site specific with lesser side effects</td>
<td>Chan et al., 1983</td>
</tr>
<tr>
<td>4-Aminobenzoyl-β-alanine</td>
<td>5-ASA</td>
<td>Azo-linkage</td>
<td>Human</td>
<td>The prodrug was site specific with lesser side effects</td>
<td>Chan et al., 1983</td>
</tr>
<tr>
<td>5-ASA</td>
<td>5-ASA</td>
<td>Azo-linkage</td>
<td>Human</td>
<td>Delivers 2 molecules of 5-ASA as compared to sulphapyridine</td>
<td>Willoughby et al., 1982</td>
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<tr>
<td>Amino acid conjugates</td>
<td></td>
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<tr>
<td>Glycine</td>
<td>Salicylic acid</td>
<td>Amide linkage</td>
<td>Rabbit</td>
<td>Absorbed from upper GIT, though metabolised by microflora of large intestine</td>
<td>Shibasaki et al., 1985</td>
</tr>
<tr>
<td>Tyrosine/methionine</td>
<td>Salicylic acid</td>
<td>Amide linkage</td>
<td>Rabbit</td>
<td>Absorbed from upper GIT, though metabolised by microflora of large intestine</td>
<td>Nakamura et al., 1992a</td>
</tr>
<tr>
<td>l-Alanine/d-Alanine</td>
<td>Salicylic acid</td>
<td>Amide linkage</td>
<td>In vitro</td>
<td>Salicylic acid-d-alanine was hydrolysed to salicylic acid by intestinal microorganisms; but salicylic acid-l-alanine showed negligible hydrolysis thereby showing enantiospecific hydrolysis</td>
<td>Nakamura et al., 1992b</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Salicylic acid</td>
<td>Amide linkage</td>
<td>In vitro</td>
<td>Primary location of hydrolysis of the prodrug was colon and the prodrug was not absorbed from the upper GIT</td>
<td>Nakamura et al., 1992c</td>
</tr>
<tr>
<td>Glycine</td>
<td>5-ASA</td>
<td>Amide linkage</td>
<td>In vitro</td>
<td>Prodrug was stable in upper GIT and was hydrolysed by cecal content to release 5-ASA</td>
<td>Jung et al., 1998</td>
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<tr>
<td>Multiple linkage conjugate</td>
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<tr>
<td>N-(2-hydroxypropyl)methacrylamide copolymer linked via amino acid and aromatic azo-containing spacers</td>
<td>9-Aminocaptothecin (9-AC)</td>
<td>Aromatic azo linkage and peptide linkage</td>
<td>In vitro</td>
<td>Under simulated large intestinal conditions it was found that azo bond reduced first, followed by peptide bond releasing free (9-AC) from the conjugate; conjugates containing leucylalanine showed high colon-specific release of 9-AC as compared to alanine containing conjugates.</td>
<td>Sakuma et al., 2001</td>
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<td>Saccharide carriers</td>
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<tr>
<td>Glucose</td>
<td>Dexamethasone/prednisolone</td>
<td>Glycossidic linkage</td>
<td>Rat</td>
<td>Dexamethasone prodrug was site specific and 60% of oral dose reached the cecum. Only 15% of prednisolone prodrug reached the cecum.</td>
<td>Friend and Chang, 1984</td>
</tr>
<tr>
<td>Glucose/galactose/cellobioside</td>
<td>Dexamethasone, prednisolone hydrocortisone, fluocortisone</td>
<td>Glycossidic linkage</td>
<td>In vitro</td>
<td>Less hydrolysis of the prodrugs was seen in contents of stomach and proximal small intestine (PSI). Hydrolysis increased in contents of Distal small intestine (DSI) and was maximum in cecal content homogenates. Galactosides hydrolysed faster than glucosides which hydrolysed faster than the corresponding cellobioside.</td>
<td>Friend and Chang, 1985</td>
</tr>
<tr>
<td>Glucose</td>
<td>Dexamethasone</td>
<td>Glycossidic linkage</td>
<td>Guinea pig</td>
<td>A dose of 0.65 μmol/kg of dexamethasone-β-D-glucoside was equally effective as 1.3 μmol/kg of dexamethasone alone.</td>
<td>Friend et al., 1991; Friend and Tozer, 1992</td>
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<tr>
<td>Glucuronide conjugates</td>
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<tr>
<td>Glucuronic acid</td>
<td>Naloxone/Nalmefene</td>
<td>Glucuronide linkage</td>
<td>Rat</td>
<td>When given to morphine dependent rats, these reversed the GIT side effects caused by morphine without causing CNS withdrawal symptoms because of activation in large intestine followed by a resultant diarrhoea which excreted the prodrug/drug.</td>
<td>Simpkins et al., 1988</td>
</tr>
<tr>
<td>Budesonide</td>
<td>Glucuronide linkage</td>
<td>Rat</td>
<td>Was found to be superior than budesonide itself for treatment of colitis.</td>
<td>Cui et al., 1994</td>
<td></td>
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<tr>
<td>Dexamethasone</td>
<td>Glucuronide linkage</td>
<td>Rat</td>
<td>A 30-fold increase in glucuronidase activity was found between DSI and cecum in normal rats. Maximum glucuronidase activity was found in normal rats, followed by colitic rats, germ free rats showed minimum glucuronidase activity.</td>
<td>Haeberlin et al., 1993</td>
<td></td>
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<tr>
<td>Menthol</td>
<td>Glucuronide linkage</td>
<td>In vitro</td>
<td>There was negligible hydrolysis of the prodrug in contents of stomach, PSI and DSI of rats. Maximum hydrolysis was seen in the cecal contents followed by colon.</td>
<td>Nolen and Friend, 1994.</td>
<td></td>
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</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Drug investigated</th>
<th>Linkage type</th>
<th>In vitro/in vivo model used</th>
<th>Performance of the prodrug/conjugate</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Sulphate conjugate</td>
<td>Ursodeoxycholic acid</td>
<td>Rat</td>
<td></td>
<td>Sulphation prevents intestinal absorption of the prodrug thereby facilitating site-specific delivery to the colon.</td>
<td>Rodrigues et al., 1995</td>
</tr>
<tr>
<td>Polymeric prodrugs</td>
<td>Dexamethasone</td>
<td>Amide linkage</td>
<td>Rat</td>
<td>Tissue/blood concentration was increased to 1.38 on administration of prodrug as against 0.55 for dexamethasone solution.</td>
<td>Leopold and Friend, 1995a,b</td>
</tr>
<tr>
<td>Sulphanilamido ethylene polymer</td>
<td>5-ASA</td>
<td>Azo linkage</td>
<td>Guinea pig/humans</td>
<td>This prodrug was more effective than sulphasalazine in reducing inflammation in guinea pig ulcerative colitis model. It has also shown benefits to patients with mild to moderately severe colitis.</td>
<td>Garretto et al., 1983; Lashner et al., 1985</td>
</tr>
<tr>
<td>Dextran conjugates</td>
<td>Naproxen</td>
<td>Ester linkage</td>
<td>Rabbit</td>
<td>Drug regeneration took place in GIT. The relative bioavailability of conjugate as compared to naproxen taken orally was 62%.</td>
<td>Harboe et al., 1988</td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>Ester linkage</td>
<td>Pig</td>
<td>Relative bioavailability of the drug was found to be unity. Bioavailability of 100% was observed.</td>
<td>Harboe et al., 1989a,b</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>Using spacer</td>
<td>Rat</td>
<td>Dexamethasone was released in the cecum and the colon and treated experimental colitis in rats.</td>
<td>McLeod et al., 1994</td>
</tr>
<tr>
<td></td>
<td>5-ASA</td>
<td>Ester</td>
<td>In vitro</td>
<td>Prodrug was stable in the upper GIT conditions and 5-ASA was liberated in contents of cecum.</td>
<td>Jung et al., 1998</td>
</tr>
<tr>
<td></td>
<td>5-ASA</td>
<td>Azo linkage</td>
<td>In vitro</td>
<td>This prodrug gave drug release comparable to sulphasalazine in the environments of the cecum and colon and showed no hydrolysis in the upper GIT conditions.</td>
<td>Schacht et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Nalidixic acid</td>
<td>Ester</td>
<td>In vitro</td>
<td>Esters with varying degree of substitution (DS) were investigated for colon specific drug delivery. These conjugates were stable at gastric and small intestinal pH. Degree of depolymerisation by dextranase decreased as DS increased. Incubation of conjugates with DS of 7 or 17% in cecal contents of rat for 24 h released 41 or 32% of nalidixic acid from the dose respectively.</td>
<td>Lee et al., 2001</td>
</tr>
<tr>
<td>Cycloextrin conjugates</td>
<td>Biphenyl acetic acid</td>
<td>Ester/amide</td>
<td>In vitro</td>
<td>Amide prodrug showed no hydrolysis in contents of stomach, small intestine and colon of rats. Ester prodrug showed 10% hydrolysis in content of stomach and small intestine but a significant hydrolysis in contents of cecum and colon. Increasing the percentage of cecal contents increased the hydrolysis.</td>
<td>Uekama et al., 1997; Minami et al., 1998</td>
</tr>
</tbody>
</table>

3.3. Polysaccharide-based delivery systems

Use of naturally occurring polysaccharides is attracting lot of attention for drug targeting to the colon since these polymers of monosaccharides are found in abundance, have wide availability, are inexpensive and are available in a variety of structures with varied properties (Hovgaard and Brondsted, 1996). They can be easily modified chemically and biochemically and are highly stable, safe, non-toxic, hydrophilic and gel forming and in addition biodegradable. These include naturally occurring polysaccharides obtained from plant (guar gum, inulin), animal (chitosan, chondroitin sulphate), algal (alginites) or microbial (dextran) origin. These are broken down by the colonic microflora to simple saccharides. So, these fall into the category of ‘generally regarded as safe’ (GRAS). An active research is going on in the field of drug targeting to the colon using these polysaccharides (Table 3).

Many of the polysaccharide-based delivery systems shield the drug from the hostile environments of the upper GIT. When these delivery systems arrive into the colon the glycosidic linkages within the polysaccharides are hydrolysed releasing the drug candidate. The main saccharolytic species are Bacteroides and Bifidobacterium.

Chitosan is a high molecular weight cationic polysaccharide, poly(N-glucosamine), derived from chitin in crab and shrimp shells by deacetylation. It is degraded by the rich colonic microflora. Chitosan has been evaluated for colon specific drug delivery mainly in the form of a capsule forming material. Enteric-coated capsules have
<table>
<thead>
<tr>
<th>Azo polymer</th>
<th>Dosage form prepared</th>
<th>Drug investigated</th>
<th>In vitro/in vivo model used</th>
<th>Summary of the results obtained</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copolymers of styrene with 2-hydroxyethyl methacrylate</td>
<td>Coating over capsules</td>
<td>Vasopressin</td>
<td>Rats</td>
<td>These capsules showed biological response characteristics of these peptide hormones in dog though it varied quantitatively.</td>
<td>Saffran et al., 1986, 1988, 1991</td>
</tr>
<tr>
<td>Hydrogel of N,N-dimethyl-acrylamide, N,N-di-butylacrylamide and acrylic acid cross-linked with azoaromatic compounds</td>
<td>Hydrogels</td>
<td>Degradation study</td>
<td>Rat</td>
<td>The degradability of the hydrogel was higher when swelling was higher and was decreased as the cross-linking density increased. Longer cross-linker degraded faster than shorter.</td>
<td>Brondsted and Kopecek, 1992</td>
</tr>
<tr>
<td>Coplymers of N-(2-hydroxypropyl)methacrylamide (HPMA) polymer with bioadhesive fucosylamine</td>
<td>Bioadhesive polymer bound to the drug</td>
<td>5-ASA</td>
<td>Guinea pig</td>
<td>Fucose containing HPMA copolymers adhered to colon of guinea pig. 5-ASA bound to the polymer was released site specifically in the colon.</td>
<td>Kopecek et al., 1992</td>
</tr>
<tr>
<td>Biodegradable pH sensitive hydrogels with enzymatically degradable azoaromatic crosslinks.</td>
<td>Biodegradable hydrogel</td>
<td>–</td>
<td>Biodegradation studies in vitro/in vivo</td>
<td>At lower pH, these showed less swelling and least drug release. As pH increased the hydrogels swell in the small intestine, further in the colon, crosslinks become accessible to azoreductase showing drug release. These were suggested to be suitable for delivery of proteins and peptides</td>
<td>Kopecek et al., 1992</td>
</tr>
<tr>
<td>2-Hydroxyethyl methacrylate, methyl methacrylate and methacrylic acid and containing N,N'-bis[methacryloyloxyethyl]oxy(carbonylamino)azobenzene</td>
<td>Coating over capsules</td>
<td>Theophylline</td>
<td>Rats</td>
<td>Though, these coatings were degraded in the colon, the degradation was slow and intersubject variation was large.</td>
<td>Van den Mooter et al., 1995</td>
</tr>
<tr>
<td>Hydrogels prepared by copolymerization of 2-hydroxyethyl methacrylate with 4-methacryloyloxy) azobenzene</td>
<td>Hydrogel</td>
<td>5-Fluorouracil</td>
<td>In vitro</td>
<td>Drug release was faster and greater in human fecal media compared to simulated gastric and intestinal fluids</td>
<td>Shanta et al., 1995</td>
</tr>
<tr>
<td>Azo network, based on acrylic backbone crosslinked with 4,4'-divinylazobenzene</td>
<td>–</td>
<td>–</td>
<td>In vitro degradation and muco-adhesion</td>
<td>It was proposed that at an optimum cross-linking density, non-adhesive particles would reach the colon where the azo network degrades and mucoushesive interaction would occur with the colonic mucosa.</td>
<td>Kakoulides et al., 1998</td>
</tr>
<tr>
<td>Segmented polyurethanes</td>
<td>Coating over pellets</td>
<td>Budesonide</td>
<td>Rat</td>
<td>These azopolymer-coated pellets were useful for colon-specific delivery of budesonide to bring healing in induced colitis.</td>
<td>Tozaki et al., 1999b</td>
</tr>
<tr>
<td>Aromatic azo bond containing urethane analogues</td>
<td>Degradable films</td>
<td>5-ASA</td>
<td>In vitro degradation of films in presence of <em>Lactobacillus</em></td>
<td>These films were degraded by azoreductase. The permeability of 5-ASA from <em>Lactobacillus</em> treated films was significantly higher than that of control</td>
<td>Chavan et al., 2001</td>
</tr>
<tr>
<td>Segmented polyurethanes with azo aromatic groups in the main chain</td>
<td>Azo polymer-coated pellets</td>
<td>Fluorescein isothiocyanate dextran (FD-4)</td>
<td>In vitro</td>
<td>Little release of FD-4 was seen in phosphate-buffered saline. Release was markedly increased in presence of rat cecal contents</td>
<td>Tozaki et al., 2001</td>
</tr>
<tr>
<td>Insulin azo hydrogels consisting of methacrylated insulin (MA-IN) copolymers with the aromatic azo agent bis[methacryloylamino]azobenzene (BMAAB) and 2-hydroxyethyl methacrylate (HEMA) or methacrylic acid (MA)</td>
<td>Hydrogels</td>
<td>Prednisolone</td>
<td>In vitro</td>
<td>Release behaviour of prednisolone from hydrogels containing MA or HEMA was the same. More than 80% drug was released during the first 3 h from MA-IN:HEMA hydrogels and around 50% drug was released from MA-IN:HEMA:BMAAB hydrogels.</td>
<td>Maris et al., 2001</td>
</tr>
</tbody>
</table>
Table 3
Polysaccharides investigated for colon-specific drug delivery with their dosage forms and summary of the results obtained

<table>
<thead>
<tr>
<th>Polysaccharide investigated</th>
<th>Drug moiety used</th>
<th>Dosage form prepared</th>
<th>In vitro/in vivo model used</th>
<th>Performance of the system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>5-(6)-Carboxy fluorescein (CF)</td>
<td>Enteric-coated chitosan capsules</td>
<td>In vitro</td>
<td>Little release of CF in upper GIT conditions and 100% drug release in 33% cecal contents within 4 h of dissolution.</td>
<td>Tozaki et al., 1997</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>Enteric-coated chitosan capsules</td>
<td>Rat</td>
<td>Chitosan capsules carried the drug to the colon. Improvement in insulin absorption seen by co-administration of absorption enhancers.</td>
<td>Tozaki et al., 1997</td>
</tr>
<tr>
<td>R68070</td>
<td></td>
<td>Enteric-coated chitosan capsules</td>
<td>Rat</td>
<td>Reduction in induced colitis was observed when R68070 was given in chitosan capsules as compared to when given in suspension</td>
<td>Tozaki et al., 1999a</td>
</tr>
<tr>
<td>Sodium diclofenac</td>
<td></td>
<td>Enteric-coated chitosan microspheres</td>
<td>In vitro</td>
<td>No drug release was seen in stomach pH and drug release completed in basic environment in 4 h</td>
<td>Lorenzo-Lamosa et al., 1998</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td>Cores coated with chitosan followed by gastric acid resistant material phytin</td>
<td>In vitro</td>
<td>Both layers together protected drug core in upper GIT conditions and drug was postulated to be released upon biodegradation of chitosan in the colon.</td>
<td>Tominaga et al., 1998</td>
</tr>
</tbody>
</table>

**Derivatives**

| Chitosan succinate          | Sodium diclofenac              | As matrices                          | In vitro                   | Reduced drug release was seen in acidic conditions and improved dissolution under basic conditions. | Aiedeh and Taha, 1999     |
| Chitosan phthalate (used as calcium salt) | Indomethacin                   | Matrices                             | In vitro                   | In the presence of rat cecal content drug release was 60.8±15.7% as compared to 4.9±1.1% in control. | Rubinstein et al., 1993   |
| Indomethacin                |                                | Compression coated/ matrix tablets    | In vitro/dogs               | In the in vitro studies release of indomethacin was increased in presence of pectinolytic enzymes and compression coated tablets of indomethacin showed better results than matrices. | Rubinstein and Radai, 1995 |
| Insulin                     |                                | Compression coated/ matrix tablets    | In vitro                   | In the in vivo studies neither of the two types of tablets could resist an initial leak of the insulin from the tablet and it was suggested that an additional protection was required for colon drug delivery. | Rubinstein and Radai, 1995 |
| Radioactive tracer          | Enteric-coated calcium pectinate matrix tablets prepared with pectin/guar as binders (Ca/P and Ca/P/GG tablets, respectively) | Human                                |                           | Intact tablets arrived into the colon and disintegrated here completely. CaP/GG tablets showed slower disintegration than CaP/P tablets. | Adkin et al., 1997        |

**Derivatives**

| Methoxylated pectinate      | Radioactive tracer             | Compression coat                      | In vitro/Human             | In vitro studies showed that compression coat could protect the drug coat in upper GIT and was degraded in the colon. These findings were confirmed in the in vivo scintigraphic studies. | Ashford et al., 1993, 1994 |
| Amidated pectin             | Paracetamol                    | Matrix tablets                        | In vitro                   | These matrices were not suitable for drug delivery to the colon. | Wakerly, 1995; Wakerly et al., 1997 |
| Indomethacin                |                                | Chitosan-coated amidated pectin beads | In vitro                   | Release of both the drugs was significantly reduced in simulated gastric and intestinal juice from the coated beads. In simulated colonic medium, both the drugs were released in around 2 h. | Munjeri et al., 1997      |
| Amidated pectin/ calcium pectinate | Ropivacaine                  | Matrix tablet with ethyl cellulose as drug matrix additive | In vitro                   | Amidated pectin were more susceptible to pectinolytic enzymes as compared to calcium pectinate. Addition of ethyl cellulose increased the tablets strength and dissolution rate. Coating this formulation with Eudragit L100 reduced drug release in upper GIT conditions without affecting enzyme degradability. | Ahrami et al., 2000       |
Table 3. Continued

<table>
<thead>
<tr>
<th>Polysaccharide investigated</th>
<th>Drug moiety used</th>
<th>Dosage form prepared</th>
<th>In vitro / in vivo model used</th>
<th>Performance of the system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin (as mixed film)</td>
<td>Paracetamol</td>
<td>Mixed film with ethyl cellulose as coating</td>
<td>In vitro</td>
<td>These films also showed colon-specific degradation. Release rate of paracetamol could be decreased by either increasing the content of ethyl cellulose in the film or by increasing the coat thickness.</td>
<td>Wakerly et al., 1996</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Mixed film with Aquacoat/ Surelease/Eudragit as coating</td>
<td>In vitro</td>
<td>Pectin was released from these films. These mixed films were not suitable for drug targeting to the colon.</td>
<td>Semde et al., 1998, 2000a,b</td>
<td></td>
</tr>
<tr>
<td>Technetium-99</td>
<td>Mixed film of pectin, chitosan and HPMC (3:1:1)</td>
<td>In vitro / Human</td>
<td>Radioactivity imaging revealed that it was concentrated in a small area till stomach and small intestine and upon reaching colon, it spread through the ascending and transverse colon indicating degradation of coating.</td>
<td>MacLeod et al., 1999a,b</td>
<td></td>
</tr>
<tr>
<td>Inter-polymer complex</td>
<td>Indomethacin/ paracetamol</td>
<td>Compression coat</td>
<td>In vitro</td>
<td>This coat provided better protection than pectin alone in upper GIT conditions. Water insoluble drug like indomethacin could be better protected by this coat in the upper GIT than water soluble drug paracetamol</td>
<td>Fernandez-Hervas and Fell, 1998</td>
</tr>
<tr>
<td>Guar gum</td>
<td>Dexamethasone/ budesonide</td>
<td>Matrix tablet</td>
<td>In vitro</td>
<td>Tablets containing 60.5% w/w of guar gum showed negligible drug release in upper GIT conditions. Presence of galactomannanase accelerated drug release.</td>
<td>Wong et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>Matrix tablet (radio labelled)</td>
<td>Human</td>
<td>Tablets designed for delayed delivery disintegrated completely in the colon and released 72–82% of drug in the colon.</td>
<td>Kenyon et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>Matrix tablet</td>
<td>In vitro</td>
<td>Only 21% of drug release was observed in upper GIT conditions. Presence of rat cecal contents increase drug release. As the concentration of cecal content was increased drug release increased further.</td>
<td>Rama Prasad et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Technetium-99m-DTPA</td>
<td>Matrix tablet</td>
<td>Human</td>
<td>Matrix tablets containing 77% guar gum released only small amount of tracer in upper GIT and bulk of tracer was released in the ascending colon.</td>
<td>Krishnaiah et al., 1998</td>
</tr>
<tr>
<td></td>
<td>5-ASA</td>
<td>Compression coat</td>
<td>In vitro</td>
<td>A guar gum coat of 150 mg showed 95.1±1.50% of 5-ASA release in presence of rat cecal content after 26 h. A guar coating between 0.61 to 0.91 mm was found sufficient to deliver the drug selectively to the colon.</td>
<td>Krishnaiah et al., 1999</td>
</tr>
<tr>
<td>Borax cross-linked guar</td>
<td>–</td>
<td>–</td>
<td>In vitro degradation in presence of galactomannase</td>
<td>Time required for degradation of these crosslinked guar showed that drug release would be in the proximal colon.</td>
<td>Rubinstein and Gliko-Kabir, 1995a,b</td>
</tr>
<tr>
<td>Phosphated cross-linked guar</td>
<td>Hydrocortisone</td>
<td>Hydrogels</td>
<td>In vitro/Rat</td>
<td>In vitro studies showed that these hydrogels were able to resist the release of 80% of drug for 6 h in phosphate buffer pH 6.4. In vivo studies showed that modified crosslinked guar showed degradation by enzymes in concentration dependent manner and was suitable for colonic drug delivery.</td>
<td>Gliko-Kabir et al., 2000</td>
</tr>
<tr>
<td>Dextran</td>
<td>Disocyanate</td>
<td>As hydrogels</td>
<td>In vitro degradation</td>
<td>These were completely degraded in human colonic fermentation model though degradation started only after 24 h.</td>
<td>Brondsted et al., 1995a; Simonsen et al., 1995</td>
</tr>
<tr>
<td></td>
<td>pH-sensitive</td>
<td>As hydrogels</td>
<td>In vitro</td>
<td>The release of BSA from these hydrogels was determined by the swelling extent. Drug release rate was enhanced by addition of dextranase in the dissolution media.</td>
<td>Chiu et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td>As capsules material</td>
<td>In vitro</td>
<td>35% drug was released in 24 h at pH 5.4. Addition of dextranase resulted in rapid degradation of capsule with fast and complete drug release.</td>
<td>Brondsted et al., 1998</td>
</tr>
</tbody>
</table>
Table 3. Continued

<table>
<thead>
<tr>
<th>Polysaccharide investigated</th>
<th>Drug moiety used</th>
<th>Dosage form prepared</th>
<th>In vitro/in vivo model used</th>
<th>Performance of the system</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran fatty acid esters (Degree of substitution 0.12–0.40)</td>
<td>Theophylline</td>
<td>As films</td>
<td>In vitro</td>
<td>Though, initial studies showed that these were suitable for colonic drug delivery, further studies showed that these were unsuitable for colonic drug delivery</td>
<td>Hirsch et al., 1997</td>
</tr>
<tr>
<td>Inulin</td>
<td>Mixed films</td>
<td>–</td>
<td>With Eudragit</td>
<td>In vitro degradation</td>
<td>Films were degraded in human fecal medium and resisted degradation in upper GIT conditions.</td>
</tr>
<tr>
<td>Methacrylated insulin</td>
<td>Cross-linked hydrogels</td>
<td>–</td>
<td>Crosslinked</td>
<td>In vitro degradation</td>
<td>Increasing the concentrations of inulinase degraded inulin faster. Increasing substitution on inulin resulted in decreased degradation.</td>
</tr>
<tr>
<td>Chondroitin sulphate</td>
<td>Cross-linked chondroitin</td>
<td>Indomethacin</td>
<td>Matrix tablet</td>
<td>In vitro</td>
<td>Drug release increase in presence of rat cecal content. Also it was observed that as cross-linking increased, drug release decreased</td>
</tr>
<tr>
<td>Amylose</td>
<td>Mixed films (Amylose Ethocel®)</td>
<td>5-ASA</td>
<td>Coated pellets</td>
<td>In vitro</td>
<td>A coating of amylose/ethylcellulose (1:4) suppressed drug release in upper GIT conditions and fermented in colonic environment.</td>
</tr>
<tr>
<td>Amylose/ethyl cellulose coating (1:4)</td>
<td>Glucose</td>
<td>Coated cores</td>
<td>Human</td>
<td>Release of glucose was delayed till the cores arrived into the cecum.</td>
<td>Cummings et al., 1996; Milojevic et al., 1996b</td>
</tr>
<tr>
<td>Organic solvent-based amylose/ethylcellulose film as coating</td>
<td>5-ASA</td>
<td>Coated pellets</td>
<td>In vitro</td>
<td>Drug release rate was inversely proportional to coating thickness. A coating containing 1:1 ratio of amylose and ethyl cellulose could resist drug release in the upper GIT and gave a rapid drug release in colonic environment.</td>
<td>Siew et al., 2000a,b</td>
</tr>
<tr>
<td>Starch</td>
<td>Radioactive tracer</td>
<td>Enteric-coated capsules</td>
<td>Human</td>
<td>These capsules have been found to be promising agents for colonic drug delivery. Varying the thickness of Eudragit could vary site of disintegration of capsules in the colon.</td>
<td>Watts and Illum, 1999; Vilivalam et al., 2000</td>
</tr>
<tr>
<td>Alginates</td>
<td>As calcium salt</td>
<td>5-ASA</td>
<td>Double coated swellable beads</td>
<td>In vitro</td>
<td>In basic media enteric coating dissolves and beads swell to exceed the strength of aquacoat film, which then burst, releasing the drug.</td>
</tr>
<tr>
<td>Locust bean gum</td>
<td>Theophylline</td>
<td>Film</td>
<td>In vitro</td>
<td>These film were mechanically unstable in the dissolution media.</td>
<td>Bauer and Kesselhut, 1995</td>
</tr>
</tbody>
</table>

been found to be quite site specific in the in vivo studies carried out in wistar rats (Tozaki et al., 1997, 1999a) (Table 3). These capsules accelerated the healing effect of R68070 (a new thromboxane synthase inhibitor) in drug induced colitis in rats.

Pectin is another non-starch linear polysaccharides with mainly α-(1–4)-linked D-galacturonic acid residues interrupted by 1,2-linked L-rhamnose residues. It remains intact in the physiological condition of the stomach and the small intestine and is degraded by the bacterial inhabitants of human colon especially by Bacteroides (Rubinstein et al., 1993; Werch and Ivy, 1941; Salyers et al., 1977). To reduce the aqueous solubility of pectin, its calcium salt has been used. Matrix tablets of calcium pectinate showed promising results in vitro (Ashford et al., 1994). For water insoluble drugs, compression coating of the core was found to be more suitable for colon drug delivery as compared to matrices (Rubinstein and Radai, 1995). However, the in vivo performance of both the types of tablets for delivery of water-soluble drug, insulin to the colon of pancreatectomized dogs showed an initial drug leak.

Dextrans consist of a linear polymer backbone with mainly 1,6-α-β-glucopyranosidic linkage. These glycosidic linkages are hydrolysed by dextranases. Dextranase activity of the colon is shown by gram negative intestinal bacteria especially the Bacteroides (Hehre and Sery, 1952). These have been used mainly as prodrugs although, other type of dosage forms have also been investigated (Table 3).

Cyclodextrins are cyclic oligosaccharides consisting of 6–8 glucose units linked through α-(1,4’)-glucosidic bonds. These are neither hydrolysed nor absorbed from the stomach and small intestine. The vast microflora present in
the colon especially, *Bacteroides* break these into small saccharides (Sinha and Kumria, 2001b). Prodrugs of these have been investigated for colonic drug delivery (Table 1).

Guar gum is a naturally occurring galactomannan polysaccharide consisting of a linear chain of \( \beta-D \)-mannopyranose joined by \( \beta-(1\rightarrow4) \) linkage with \( \alpha-D \)-galactopyranosyl units attached by 1,6-links in the ratio of 1:2. It is susceptible to microbial degradation in the large intestine (Bayliss and Houston, 1986; Tomolin et al., 1989; Macfarlane et al., 1990). Guar gum has also been investigated as a matrix tablet for delivery of water insoluble drugs to the colon. These tablets have shown promising results in vitro and in vivo (Kenyon et al., 1997; Rama Prasad et al., 1998) (Table 3). Another study using compression coating has also shown the suitability of this polymer for the above purpose (Krishnaiah et al., 1999).

Inulin is another naturally occurring polysaccharide found in many plants, such as onion, garlic, chicory, artichoke. Chemically, it consists of \( \beta-2-1 \) linked \( \alpha-D \)-fructose molecules, having a glucosyl unit at the reducing end (Roberfroid, 1993). It is not hydrolysed by secretions of the human digestive tract (Dysseler and Hoffem, 1995). Bacteria present in the colon especially *Bifidobacteria*, which constitute up to 25% of the normal gut flora in man (McKellar and Modler, 1989) are known to ferment inulin (Wang and Gibson, 1993; Gibson and Roberfroid, 1995). Hydrogels of inulin have been investigated as colon drug carriers (Table 3). Recently azo crosslinked inulin has been investigated as a colonic drug carrier (Table 2).

Starch has been evaluated as a carrier for colon specific drug delivery as enteric-coated capsules. These are advantageous over the conventional gelatin capsules, due to colon specific degradation of these capsules (Vililvalam et al., 2000).

Amylose is a constituent of starch consisting of \( D \)-glucopyranose residues linked by \( \alpha-(1\rightarrow4) \) bond. These are resistant to pancreatic \( \alpha \)-amylase but are degraded by colonic bacterial enzyme (Englyst and MacFarlane, 1986; Ring et al., 1988). Mixed film of amylose with ethyl cellulose as coatings have shown to have a great potential as colon delivery carriers.

Chondroitin sulphate is a mucopolysaccharide found in animal connective tissue. It consists of \( D \)-glucuronic acid linked to \( D \)-acetyl-\( D \)-galactosamine, which is sulphated at C-6. It is degraded by the anaerobic bacteria of the large intestine mainly by *Bacteroides thetaiotaomicron* and *B. ovatus* (Salyers, 1979; Salyers and Brien, 1980). Tablets prepared using cross-linked chondroitin sulphate have been found to be suitable for colonic delivery of water insoluble drug indomethacin (Rubinstein et al., 1992b).

Though a number of polysaccharides are available for colon specific drug delivery, a usual problem encountered with them is their high water solubility. The high water solubility and poor film forming property is disadvantageous and would lead to premature drug release in the tracts of the upper GIT. One approach that may alter the solubility of the polysaccharide is chemical derivatization or crosslinking. A balance between the derivatization/ crosslinking and solubility reduction is maintained so that the derivatised or crosslinked polysaccharide does not reduce the biodegradability. To overcome the poor film forming property of these polysaccharides these are mixed with other synthetic film forming polymers to form mixed films. These are again prepared using care that the films resist drug release in the tracts of the upper GIT but retain the bacterial degradability (Milojevic et al., 1996a,b).

4. Conclusion

The GIT has for many reasons been the most popular route for drug delivery despite some known disadvantages. Moreover, the current trend in oral therapy is to explore possibilities for targeted drug delivery within the GIT. Colon, which is the terminal part of the GIT has gained recognition in recent years as a site for drug delivery for various drugs including novel therapeutic drugs, i.e. peptides.

All the reported methods of drug delivery to the colon are more or less susceptible to changes in the diet and to environmental variables. This questions their reliability. The activity of the microbial enzymes is even more susceptible to diet, drug intake (particularly antibiotics and certain laxatives) and environmental factors. However, as the commonly exploited enzymes for the development of colon specific delivery systems such as azo reductase and various glycosidases are present only in the terminal ileum and the colon, premature drug release does not occur. These systems however, should be carefully designed and should be degradable by widespread colonic bacterial species. Use of these systems in diseased GIT conditions, which disturb the normal bacterial flora, needs to be investigated.

Bacterial enzyme degradable polymers need to be optimized to improve their film forming properties, their swelling behaviour and their degradability by colonic enzymes. Moreover, use of azo polymers and other synthetic polymers needs a detailed toxicological study to be performed before being used commercially. Naturally occurring polysaccharides with no major problem of toxicity can be used as biodegradable coating, as carriers for prodrugs, for the formation of matrix films, and as matrices for colon specific drug delivery. Development of polysaccharide with moieties that bind to specific regions of the intestine, such as the inflamed colonic lesions or colon cancer tissue, represent another promising approach for the treatment of local pathologies.

Acknowledgements

The Financial support of CSIR, New Delhi, India to Rachna Kumria is gratefully acknowledged.
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