Pharmaceutical Research, Vol. 18, No. 5, 2001

Review Article

Colonic Drug Delivery: Prodrug Approach

V. R. Sinha[1,2] and Rachna Kumria[1]

Received October 11, 2000; accepted January 22, 2001

The colon is largely being investigated as a site for administration of protein and peptides, which are degraded by digestive enzymes in the upper GIT. Also for local diseases of the colon, drug administration to the site of action can not only reduce the dose to be administered, but also decrease the side effects. One of the approaches used for colon specific drug delivery is the formation of a prodrug which optimizes drug delivery and improves drug efficacy. Many prodrugs have been evaluated for colon drug delivery. These prodrugs are designed to pass intact and unabsorbed from the upper GIT and undergo biotransformation in the colon releasing the active drug molecule. This biotransformation is carried out by a variety of enzymes, mainly of bacterial origin present in the colon (e.g. azoreductase, glucuronidase, glycosidase, dextranase, esterase, nitroreductase, cyclodextranase, etc.). The present review includes varius prodrug approaches investigated for colon drug delivery and their site specificity.

KEY WORDS: colon drug delivery; colon specific drug delivery; prodrugs; conjugates.

INTRODUCTION

A prodrug is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation *in vivo* to release the active drug. It should have improved delivery properties over the parent drug molecule. Site specific drug delivery through site specific prodrug activation may be accomplished by the utilization of some specific property at the target site, such as altered pH or high activity of certain enzymes relative to the non-target tissue, for the prodrug-drug conversion.

For the treatment of Inflammatory Bowel disease (IBD), with anti-inflammatory agents, varius approaches have been used to target the drug molecules to the colon. These approaches have also been used to target various other drug molecules to the colon to improve the absorption characteristics of these drugs. These include coating with biodegradable polymers, coating with pH-sensitive polymers, time-dependent formulations, forming biodegradable matrices, and forming a prodrug. All these approaches attempt to lower the absorption and release of the drug in the stomach and small intestine and thereby facilitate quantitative drug delivery to the colon.

The bacterial microflora of the stomach and the small intestine is of the order of 10^3–10^4 CFU/ml consisting mainly of gram-positive facultative bacteria (1,2). In contrast, flora of the colon is many times greater and is of the order of 10^{11}–10^{12} CFU/ml consisting of mainly anaerobic bacteria (3) (e.g. *Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria,* etc.). This vast microflora fulfills its energy needs by fermenting various types of substrates that have been left undigested in the small bowel (e.g. di- and polysaccharides, mucopolysaccharides etc.) (4). For this fermentation, the microflora produces an extensive number of enzymes like azoreductase, β-galactosidase, β-xylosidase, nitroreductase, glycosidase deaminase, etc. (5). These enzymes of the colon are largely being exploited for colon specific drug delivery. This is because these enzymes act as triggers for releasing drug molecules from various undigested drug carrier systems. The prodrug approach also exploits these enzymes for prodrug to drug conversion. Long transit time encountered in the colon (6) also gives sufficient time for action of these enzymes on the prodrug substrates.

Medical therapy for IBD is restricted to aminosalicylates, corticosteroids, and immunosuppressants. When synthesizing prodrugs, the choice of carrier depends on the functional group available on the drug molecule for conjugation with the carrier (e.g., the hydroxyl group present on the corticosteroids can enter into a glycosidic linkage (7) with various sugars, the carboxyl group of biphenylyl acetic acid forms an ester/amide conjugate with cyclodextrin (8,9) etc.). Various types of prodrugs with drug molecules linked to different carriers have been prepared and evaluated as colon-specific drug delivery agents. The parameters evaluated include absorption and stability of the prodrug in upper GIT (hydrolysis in various GIT segments, both in GIT contents and GIT tissues), selectivity of hydrolysis by the enzymes of the colon, and the amount of drug regenerated in the colon.

Amino-acid, glycoside, glucuronide, azo, dextran, and cyclodextrin conjugates are some of the conjugates evaluated for colon-specific delivery. Generally, a prodrug is successful as a colon drug carrier if it is hydrophilic and bulky to minimize absorption from the upper GIT, and if once in the colon, it is converted into a more lipophilic drug molecule, which is then available for absorption.

[1] University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India.

[2] To whom correspondence should be addressed. (e-mail: omamchd@glide.net.in)

AMINO-ACID CONJUGATES

Proteins and their basic units (i.e. the amino-acids (A.A.)) have polar groups like the $-NH_2-$ and $-COOH-$. These polar groups are hydrophilic and reduce the membrane permeability of A.A and proteins. Nakamura *et al.* (10–13) studied the conjugation of drug molecule to these polar A.A and prepared prodrugs for colon-drug delivery. Various non-essential amino acids such as glycine, tyrosine, methionine, L-alanine, glutamic acid, etc. were conjugated to salicylic acid. The salicyluric acid (i.e. the glycine conjugate of salicylic acid) (Fig. 1a) was found to be metabolized by the micro-organisms of the intestinal flora of rabbit and dog to salicylic acid. However, this prodrug was absorbed into the circulation from the upper GIT and was therefore unsuitable as a colon drug carrier. Improving the physicochemical properties of the prodrug, Nakamura *et al.* increased the hydrophilicity and length of the carrier amino acid, decreasing the membrane permeability of the conjugate, and prepared salicyclic-glutamic acid conjugates (10) (Fig. 1b). This conjugate gave good results as a colon-specific carrier for salicyclic acid. It showed minimal absorption and degradation in the upper GIT and showed more enzymatic specificity for hydrolysis by colonic enzymes. Also the drug showed maximum and sustained absorption from colon. Nakamura *et al.* also observed a significant inhibition in the formation of salicylic acid from the conjugate in rabbits pretreated with kanamycin, indicating that these derivatives need hydrolysis by the intestinal microorganism to release the parent drug moiety (12).

To study the enantioselective metabolism of the intestinal microflora, the two enantiomeric prodrugs of salicylic acid (i.e. salicylic acid-L-alanine and salicyclic acid-D-alanine) were prepared (13). On oral and intracecal administration, though, salicylic acid-L-alanine was metabolized to salicylic acid by the intestinal microflora of the rabbits; salicylic acid-D-alanine showed negligible conversion to salicylic acid. This showed the enantioselectivity of the intestinal enzymes. However, further studies are required to confirm this selectivity.

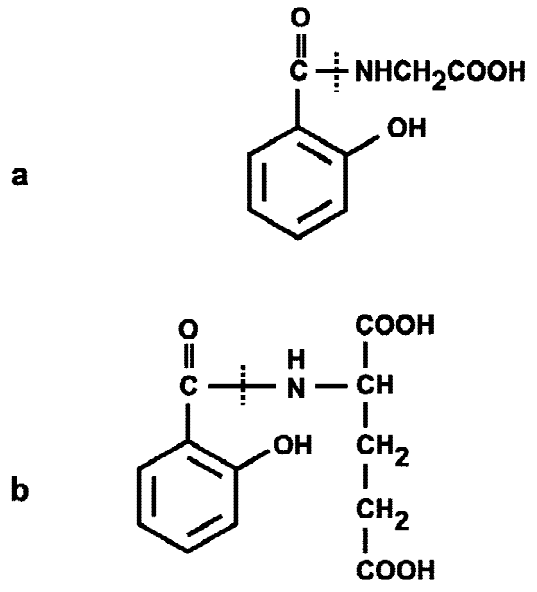

Fig. 1. Glycine and glutamic acid conjugates of salicylic acid. (a) Salicyluric acid. (b) Salicyl-glutamic acid conjugate. (Dotted line shows the site of cleavage.)

Jung *et al.* (14) synthesized 5-aminosalicyl-glycine as a prodrug for colon specific drug delivery of 5-Amino salicylic acid i.e., 5-ASA (Fig. 2). *In vitro* studies carried out in GIT contents of rat showed no release of 5-ASA in the contents of stomach and small intestine, indicating the stability of the prodrug in the upper intestine. The amount of 5-ASA liberated after incubation of prodrug was about 27% in colonic contents and 65% in cecal contents after 8 h. This indicated that prodrug activation took place primarily in the rat cecum. When cecal and colonic contents of rats pretreated with kanamycin were used, a very low concentration of 5-ASA was observed.

The ability of Poly-(L-Aspartic acid) to act as a carrier for dexamethasone was also investigated (15,16). The ester prodrug with 10% w/w drug loading was synthesized using dicyclohexylcarbodiimide as dehydrating agent in dimethylformamide. *In vitro* studies indicated that maximum hydrolytic activity for this prodrug was found in the contents of cecum and colon (15). It was also observed that the hydrolytic activity of mucosa and muscle tissues of GIT was much lower as compared to the GIT contents. Studies carried out to observe hydrolytic activity in normal, colitic, and germ free rat, showed that maximum hydrolytic activity was found in normal rat followed by colitic rat. Germ-free rats showed minimum hydrolytic activity. The extent of prodrug hydrolysis in contents of large intestine of rats showed that microorganisms of the large intestine are able to hydrolyze the prodrug. For *in vivo* testing of the conjugate, it was given to the rat and results obtained were compared to a solution of dexamethasone, both administered orally (16). Tissue/blood concentration ratio in the cecum after administration of the prodrug was 1.38, where as it was 0.55 for dexamethasone solution. This signifies the advantage of the prodrug to significantly lower the blood concentration but still give a higher drug concentration at the site of action, i.e. the tissues. This study indicates that the Poly L-(Aspartic acid) back bone protects the prodrug ester bond against hydrolysis by the host digestive enzymes. The polymer backbone of poly (L-Aspartic) cleaves enzymatically into smaller fragments to allow esterases access to the polymer-drug ester bond, releasing the drug in the tissue compartment.

The amino acid carriers have been found to increase the bioavailability and efficacy of drugs and at the same time reduce their toxicity. Therefore these agents can be used for drug delivery to the large bowel for IBD and other large bowel disorders.

GLYCOSIDE CONJUGATES

Certain drugs can be conjugated to different sugar moieties to form glycosides. The drug part forms the aglycon and is linked to the sugar part, which forms the glycon part of the

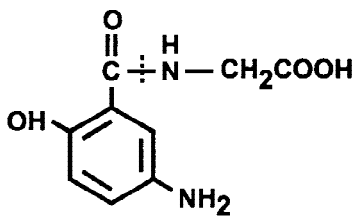

Fig. 2. 5-aminosalicyl-glycine conjugate (14).

glycoside. Various naturally occurring glycosides, e.g. the sennosides, used for laxative action have been used for ages. When taken orally, intact sennosides are more efficient as laxatives than sugar free aglycones. These sennosides are activated by colonic microflora to generate rhein anthrones, which gives the desired laxative effect (17).

The glycosides may be glucoside, galactoside, or cellobioside depending upon whether the sugar moiety is a glucose, galactose, or cellulose respectively. Because they are bulky and hydrophilic, these glycosides do not penetrate the biological membranes upon ingestion (18). They breakdown upon action of glycosidases, releasing the drug part from the sugar. Glycosidase activity of the GIT is derived from anaerobic microflora in the large bowel or the sloughed or exfoliated cells of the small intestine (19,20). The presence of glycosidase activity in the small intestine could pose a problem in delivery of these conjugates to the large bowel, because some hydrolysis of the conjugate can be expected in the small intestine. However, the small intestinal transit time, when compared to the large intestinal transit time, is short, and moreover, considering the time required for the hydrolysis of glycosidic bond, these conjugates can be expected to be good colon specific drug carriers. The major glycosidase enzymes produced by the intestinal microflora are β-D-galactosidase, α-L-arabinofuranosidase, β-D-xylopyranosidase, and β-D-glucosidase (20).

Friend and Chang (7) prepared dexamethasone-21-β-glucoside (Fig. 3) and prednisolone-21-β-glucoside for delivery of these steroids to the colon. The results of prodrug administration were compared to administration of free steroids, both given orally. When given as free steroids, the drug was extensively absorbed from the small intestine and less than 1% of either steroid reached the cecum. On the contrary, *in vivo* studies on dexamethasone-21-β-glucoside showed that nearly 60% of the oral dose reached the cecum between 4–5 h. Here the prodrug was rapidly hydrolyzed showing 44% of dose administered as free steroid in the cecum at the fifth hour. Difference between 60% of prodrug in the colon and 44% of drug found was due to the absorption of free steroid from the cecum. The prednisolone prodrug was not that site-specific, with 15% of dose administered reaching the cecum in 4–5 h and only 11% recovered unabsorbed from the cecum.

Comparing the efficacy of different sugar moieties to act as carriers for corticosteroids, Friend and Chang (21) prepared glucosides, galactosides, and cellobiosides of dexamethasone, prednisolone, hydrocortisone, and fludrocortisone. *In vitro* studies of these glycosides were conducted in homogenates of different segments of the GIT. Hydrolysis of all

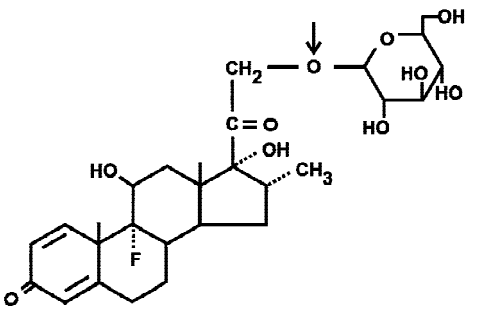

Fig. 3. Dexamethasone-21-β-D-glucoside (7). (Arrow shows site of action of glycosidase.)

these prodrugs was low in the stomach and the proximal small intestinal (PSI) contents. The hydrolysis increased in the contents of distal small intestine (DSI) and was highest in the cecal content homogenates. Galactoside prodrugs were found to be more rapidly hydrolyzed than the corresponding glucosides which hydrolyzed faster than the cellobioside.

In vitro studies carried out specifically on dexamethasone-β-D-glucoside (22,23) showed that both GIT tissues and GIT contents of guinea pig showed β-glucosidase activity. Among the tissues, maximum activity was seen in the tissues of PSI whereas among the contents maximum activity was seen in the cecum and the colon. *In vivo* studies were carried out in IBD model of carrageenan induced ulcers in guinea pigs which showed that 0.65 μmol/kg dose of dexamethasone-β-D-glucoside was equally effective as 1.3 μmol/kg of dexamethasone alone. This suggested that by using the prodrug, side effects can be reduced without compromising the efficacy. The bioavailability of dexamethasone from its glucoside prodrug was found to be unity.

GLUCURONIDE AND SULPHATE CONJUGATES

Glucuronide and sulphate conjugation are the major mechanisms for the inactivation and preparation for clearance of a variety of drugs. Bacteria of the lower GIT, however, secrete β-glucuronidase (24) and can deglucuronidate a variety of drugs in the intestine. Thus, the deglucuronidation process results in the release of the active drug again and enables its reabsorption. Considering this, glucuronide prodrugs can be expected to be superior agents for drug delivery to the colon.

Opiates, when taken for the relief of pain, cause severe constipation by inhibiting GIT motility and secretions. Narcotic antagonists, when given as antidotes for GIT side effects, immediately relieve constipation but precipitate acute withdrawal. This is because these narcotic antagonists are not selective and they not only effect the GIT activity, but also the central nervous system (CNS). A novel approach would be to target these antagonists to the lower bowel so that they are not absorbed systemically. With this purpose, naloxone and nalmefene-glucuronide prodrugs were prepared to target these drugs to the colon (25). When given orally to morphine-dependent rats these prodrugs showed increased GIT motility and secretion in the large bowel causing a resultant diarrhea and signifying that drug regeneration took place in the regions of colon. This was also indicated by the transit time and intracecal administration of the prodrug. The resultant diarrhea flushed out the drug/prodrug from the colon thereby preventing the systemic absorption of the antagonist, which in-turn caused absence of withdrawal symptoms.

A similar glucuronide prodrug of dexamethasone, namely dexamethasone-β-D-glucuronide, was prepared by Haeberlin *et al.* (26) (Fig. 4). They conducted a study on conventional, colitic, and germ free rats and found that there was a 30-fold increase in luminal β-D-glucuronidase activity between DSI and cecum in normal rats. The glucosidase activity gradient between DSI and cecum is much lower, suggesting that glucuronide prodrugs can deliver the drugs more specifically to the colon and are less susceptible to premature hydrolysis in stomach and small intestine as compared to the respective glucoside prodrug. Also, it was observed that maximum hydrolytic activity was in the luminal contents of con-

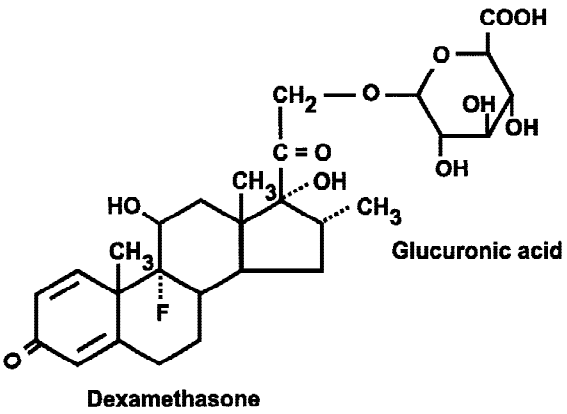

Fig. 4. Dexamethasone-β-D-glucuronide.

ventional rats followed by colitic rats. Germ free rats showed very low luminal β-D-glucuronidase activity.

Budesonide-β-glucuronide prodrug was also found to be superior to budesonide itself for the treatment of colitis in the rat (27). Also the side effect of adrenal suppression observed on administration of these steroids was lacking when the conjugate was given. A similar study conducted in normal and colitic rats showed a high level of drug delivery to the colon using glucuronide prodrugs of dexamethasone and budesonide (28).

Peppermint oil has been found to control the large intestinal spasm and motility and is therefore used for irritable bowel syndrome (IBS). The primary component of peppermint oil, menthol, is rapidly absorbed when given orally and does not reach the lower bowel, its site of action. Menthol-β-glucuronide prodrug, having a larger size and a decreased partition co-efficient as compared to menthol, has a substantially limited absorption in the GIT. This prodrug shows negligible hydrolysis in luminal contents of rats, stomach, PSI, and DSI (29). The hydrolytic activity was maximum in the cecum followed by colon. So, this prodrug can be used for the specific treatment of IBS.

Ursodeoxycholic acid (UDCA), primarily used for the treatment of liver diseases, has been found to have a protective role in colonic carcinogenesis. It has also been found to cause reduction in incidence of colonic tumours. But when UDCA is given orally, it is absorbed from the intestine and is biotransformed in the liver and does not reach the colon. However, Rodrigues *et al.* (30) found that sulfation of UDCA increases the hydrophilicity of the molecule and prevents its absorption from the intestine thereby facilitating colonic delivery.

AZO-CONJUGATES

The azo linkage exhibits a wide range of thermal, chemical, photochemical, and pharmaceutical properties. These azo compounds are extensively metabolized by the intestinal bacteria, both by intracellular enzymatic component and extracellular reduction (31). The use of these azo compounds for colon-targeting has been in the form of hydrogels as a coating material for coating the drug cores and as prodrugs (32). In the latter approach the drug is attached via an azo bond to a carrier. This azo bond is stable in the upper GIT and is cleaved in the colon by the azo-reductases produced by the microflora.

Sulphasalazine, which was used for the treatment of rheumatoid arthiritis, was later known to have potential in the treatment of IBD (33). This compound has an azo bound between 5-ASA and sulphapyridine (SP) (Fig. 5a). In the colon, the azoreductases cleave the azo bond releasing the drug, 5-ASA and the carrier SP. With the knowledge that the adverse effects associated with sulphasalazine are due to SP, an investigation started for the choice of a suitable carrier for 5-ASA with minimum adverse effects. SP was replaced by p-aminohippurate in ipsalazide and by 4-aminobenzoyl-β-alanine in balsalzide. In another approach two molecules of 5-ASA have been joined together to form an ultimate prodrug disodium azodisalicylate (olsalazine), in which one molecule of 5-ASA is used as a carrier for the other (Fig. 5b). Under normal intact GIT conditions and bacterial flora, olsalazine delivers twice the amount of 5-ASA as compared to sulphasalazine (34).

Recently newer approaches have been introduced in which polymers are azo linked to various drug molecules. These have been discussed under polymer prodrugs.

POLYMERIC PRODRUGS

Polymeric prodrugs with drug molecule linked directly to a high molecular weight polymeric backbone have also been investigated for colon-drug delivery. The linkage between the drug and the polymer is susceptible to enzymatic attack in the large intestine and the drug is released at this site.

A polymeric prodrug of 5-ASA susceptible to azoreductase was developed (35). In this prodrug sulphasalazine was linked to a non-absorbable sulphaniamidoethylene polymer to form poly-ASA (Fig. 6), which was found to be more effective than sulphasalazine in reducing the inflammation in guinea pig ulcerative colitis model. Moreover, this prodrug lacked the adverse effects associated with the cleavage product of the prodrugs as the cleavage product (i.e. SP), which is linked to the polymer and is excreted unabsorbed in the feces. This product poly-ASA has also shown clinical benefits for patients with mild to moderately severe colitis (36,37).

Kopecek *et al.* (38,39) prepared polymeric prodrugs of 5-ASA azo linked to *N*-(-hydroxypropyl)methacrylamide copolymers. These conjugates were made mucoadhesive by incorporating fucosylamine pendent moieties to increase colonic residence time of the prodrug, which would facilitate its total hydrolysis. These adhered to the mucosal lecithins. Studies showed that these prodrugs had good potential to act as carriers for colon specific drug delivery.

Polymeric systems that swell minimally under acidic con-

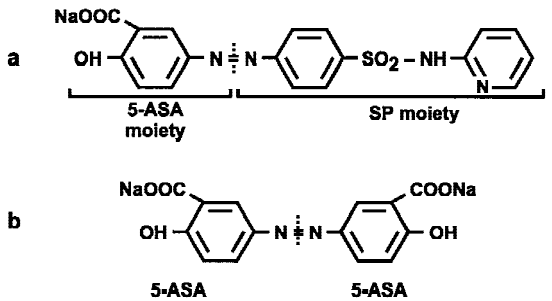

Fig. 5. Sodium salt of a. Sulphasalazine and b. Olsalazine prodrugs of 5-ASA (33).

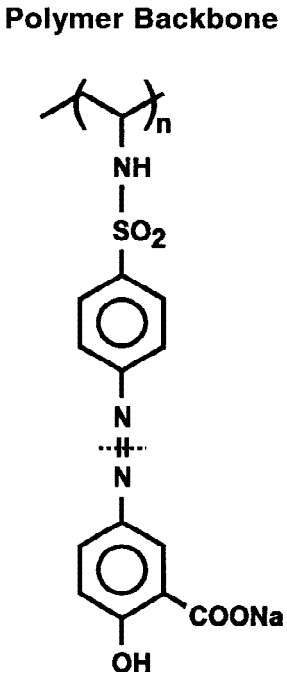

Fig. 6. Polymeric prodrug of 5-ASA (Poly-ASA) (35).

ditions in the stomach, but swell extensively under basic conditions in the large intestine, were also thought to be possible drug carriers for colon drug delivery (40). Swelling under basic conditions of the large intestine would make these polymers prone to enzymatic and hydrolytic degradation. Therefore, polymeric prodrugs of 5-ASA, namely methacryloyloxyethyl 5-aminosalicylate (MOES) and *N*-methacryloylamido ethyl-5-amino salicylamide (MAES), were prepared as monomers and polymerized (41). Due to low swelling in acidic media, these polymers could shield the drug in the stomach. As the pH increased in the GIT, these polymers swelled and became susceptible to hydrolysis. These were also found to be suitable drug carriers for colon delivery. However, the low pH-gradient between the small and large intestine makes them less site-specific and the drug release may occur in the ileum region.

Although many of these polymeric prodrugs can deliver drugs successfully to the colon, the selection of a drug candidate is an important criterion because drug weight cannot be more than 20% of the total weight of the drug carrier system. So, drugs requiring large doses cannot be carried by these systems.

CYCLODEXTRIN CONJUGATES

Cyclodextrins have been used as pharmaceutical carriers due to their stability against non-enzymatic and enzymatic degradation in various body fluids, biocompatibility, and safety profile. The α- and β-cyclodextrins are practically resistant to gastric acid, salivary, and pancreatic amylases. A clinical study has shown clear evidence that β-cyclodextrin is poorly digested in the small intestine but is almost completely degraded by the colonic microflora (42). Colonic bacteria are capable of degrading cyclodextrins for carbon source by stimulating cyclodextranase activity. They are fermented by the colonic microflora to form small saccharides that are then absorbed (42,43). This susceptibility to degradation specifically by colonic microflora together with their property to form inclusion complexes with various drugs makes them particularly useful in carrying drug moieties to the colon.

Cyclodextrin conjugates were prepared by substituting one primary hydroxyl group of the cyclodextrin with biphenylylacetic acid forming amide/ester conjugate (8,9,44) (Fig. 7). Amide conjugate showed no hydrolysis in contents of stomach, small intestine, cecum, and colon of rats. The ester conjugate showed less than 10% of drug release in the contents of stomach, small intestine and their tissue homogenates, but a significant hydrolysis in contents of cecum and colon. Increasing the percentage of cecal contents from 1% to 6.7% in the dissolution medium increased the hydrolysis of the conjugate from about 20% to 46% in 48 h. This study indicates that cyclodextrin prodrugs have a great potential as colonic drug carriers due to their stability and site-specificity.

DEXTRAN CONJUGATES

Dextrans are polysaccharides of bacterial origin where the monosaccharides are joined to each other by glycoside linkages. These linkages are hydrolysed by moulds (45), bacteria (46), and mammalian cells (47). The enzyme responsible for the hydrolysis of these linkages is dextranase. The dextranase activity is almost absent in the upper GIT, whereas high dextranase activity is shown by anaerobic gram-negative bacteria, especially the Bacteroides (48), which are present in a concentration as high as 10^{11} per gram in colon. This led to the use of dextran as carriers for drug molecules to the colon. In the colon, dextran's glycosidic bonds are hydrolyzed by dextranases to give shorter prodrug oligomers, which are further split by the colonic esterases (49–51) to release the drug free in the lumen of the colon.

Harboe *et al.* (51) carried out a series of studies on various dextran-drug conjugates. A study on dextran-naproxen ester conjugate carried out in rabbits showed that drug regeneration took place in the GIT by action of one or more enzyme systems. In the rabbit the relative bioavailability of the conjugate, as compared to naproxen taken orally, was 62%. A similar study carried out in pigs showed a relative bioavailability of 90% (52).

Further studies showed that the intact conjugate is not absorbed systemically and that the dextran backbone protects the drug from enzyme attack (50–53). These studies also revealed that drug regeneration occurs in the region of cecum and colon. The bioavailability of naproxen from dextran prodrugs was found to be nearly 100%. Harboe et al. showed the potential of dextran prodrugs for colon-specific delivery of drugs containing a carboxylic acid function (–COOH).

Glucocorticoids do not possess –COOH group so these were linked to dextran using spacer molecule (Fig. 8), Studies

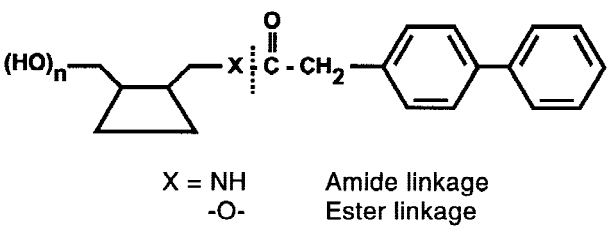

Fig. 7. Cyclodextrin-Biphenylacetic acid conjugate (9).

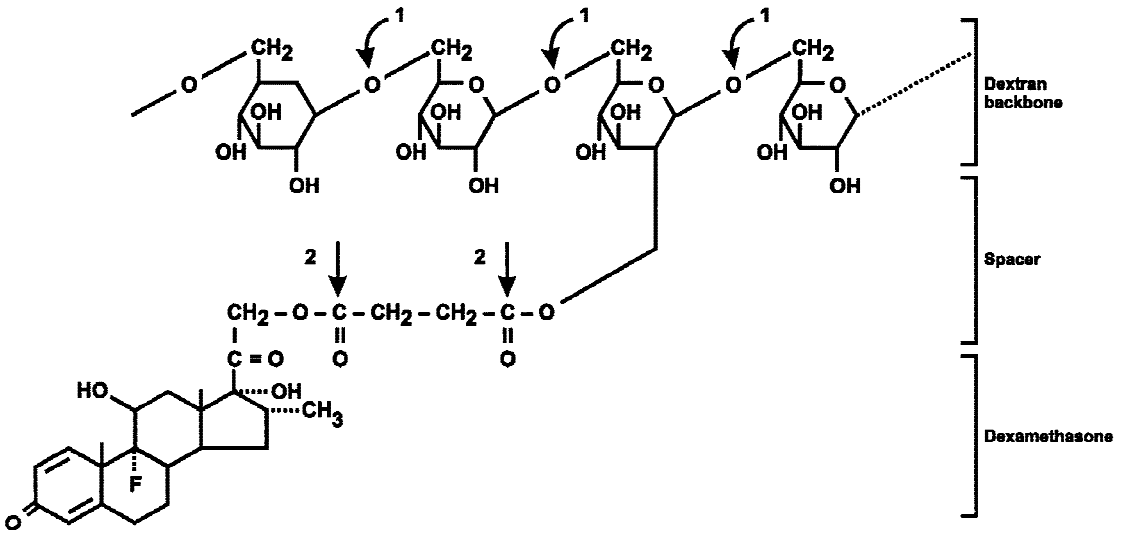

Fig. 8. Dexamethasone-Dextran conjugates with succiniate spacer (54). Arrow 1 shows site of action of dextranase. Arrow 2 shows site of action of esterases.

on glucocorticoid-dextran conjugates showed the ability of dextran to shield drug absorption/hydrolysis in the small intestine. In the cecum and colon, the glucocorticoid was released and treated the experimental colitis in rat while causing little adrenal suppression (54,55).

In a similar approach, 5-ASA was linked via a spacer molecule to three different polymeric systems, namely, poly (1-vinyl-2-pyrrolidone-co-maleic anhydride) (PVP-MA), poly [N-(2-hydroxyethyl)-DL-aspartamide (PHEA) and dextran (56). Release studies were carried out in a SHIME reactor (57). In this reactor each GIT segment was represented by a reactor that had environment simulating its respective GIT segment. All three polymeric systems could shield the drug in the environments of upper GIT and no hydrolysis of the prodrug was observed. However, PVP-MA and PHEA did not show the required hydrolysis in the reactor simulating the environments of the cecum and colon. But the dextran prodrug gave drug release comparable to that shown by sulphasalazine in the environment of cecum and colon. This is because dextran backbone breaks down on reaching the colon by action of dextranase, which then makes the drug-polymer bond accessible to hydrolysis.

More recently, Dextran-5ASA ester conjugates were also found to be stable in the upper GIT of rat (58). Liberation of 5-ASA was observed in the cecal contents suggesting the respective protective and selective action of dextran for colon-specific drug delivery.

CONCLUSION

Prodrugs seem to be promising therapeutic agents for the management of diseases of the lower bowel due to their ability to show the required action with lower doses as they release the entire dose at the site of action. Additionally, they reduce the side effects compared to the parent drug. Such systems can be formulated in a much easier manner and many technical difficulties faced in preparation of other types of colon specific delivery systems, like coated, multiple coated, systems etc., can be avoided. These agents, however, are new chemical entities and require more detailed toxicologic studies before they can be used as colon carriers. Also the biologic effects of the various carrier molecules need to be investigated further. The use of natural polysaccharide carriers, like dextrans and cyclodextrins, seems to be a better alternative than the synthetic polymers because of their *in vivo* biodegradation to simple saccharides/sugars at this time.

ACKNOWLEDGMENTS

We would like to express our thanks to University Grants Commission, New Delhi, India for providing the funds and to Dr. P. D. Sharma, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh for his valuable suggestions.

REFERENCES

1. S. L. Gorbach. Intestinal microflora. *Gastroenterology* **60**:1110–1129 (1971).
2. G. I. Simon and S. L. Gorbach. The human intestinal microflora. *Dig. Dis. Sci.* **31**:147S–162S (1986).
3. W. E. C. Moore and L. V. Holdeman. Discussion of current bacteriologic investigations of the relationships between intestinal flora, diet and colon cancer. *Cancer Res.* **35**:3418–3420 (1975).
4. A. Rubinstein. Microbially controlled drug delivery to the colon. *Biopharm. Drug Dispos.* **11**:465–475 (1990).
5. R. R. Scheline. Metabolism of foreign compounds by gastrointestinal microorganisms. *Pharmacol. Rev.* **25**:451–523 (1973).
6. R. J. Mrnsy. The colon as a site for drug delivery. *J. Control. Release* **22**:15–34 (1992).
7. D. R. Friend and G. W. Chang. A colon-specific drug delivery system based on drug glycosides and the glycosidases of colonic bacteria. *J. Med. Chem.* **27**:261–266 (1984).
8. K. Uekama, K. Minami, and F. Hirayama. 6^{A}-O[(4-biphenylyl)acetyl)]-α-,-β-, and -γ-cyclodextrins and 6^{A}-deoxy-6^{A}-[[(4-biphenylyl)acetyl] amino]-α-,-β- and -γ-cyclodextrins: Potential prodrugs for colon specific delivery. *J. Med. Chem.* **40**:2755–2761 (1997).
9. K. Minami, F. Hirayama, and K. Uekama. Colon specific drug delivery based on cyclodextrin prodrug: Release behaviour of biphenylyl acetic acid from its cyclodextrin conjugates in rat intestinal tract after oral administration. *J. Pharm. Sci.* **87**:715–720 (1998).

10. J. Nakamura, K. Asai, K. Nishida, and H. Sasaki. A novel prodrug of salicylic acid, salicylic acid-glutamic acid conjugate utilizing hydrolysis in rabbit intestinal microorganisms. *Chem. Pharm. Bull.* **40**:2164–2168 (1992).
11. J. Nakamura, M. Kido, K. Nishida, and H. Sasaki. Hydrolysis of salicylic acid-tyrosine salicylic acid-methionine prodrugs in rabbits. *Int. J. Pharm.* **87**:59–66 (1992).
12. J. Nakamura, M. Kido, K. Nishida, and H. Sasaki. Effect of oral pretreatment with antibiotics on the hydrolysis of salicylic acid-tyrosine and salicylic acid methionine prodrugs in rabbit intestinal microorganisms. *Chem. Pharm. Bull.* **40**:2572–2576 (1992).
13. J. Nakamura, C. Tagami, K. Nishida, and H. Sasaki. Unequal hydrolysis of salicylic acid-D-alanine and salicylic acid-L-alanine conjugate in rabbit intestinal microorganisms. *Chem. Pharm. Bull.* **40**:547–549 (1992).
14. Y. J. Jung, J. S. Lee, H. H. Kim, Y. M. Kim, and S. K. Han. Synthesis and evaluation of 5-aminosalicyl-glycine as a potential colon specific prodrug of 5-aminosalicyclic acid. *Arch. Pharmacal. Res.* **21**:174–178 (1998).
15. C. S. Leopold and D. R. Friend. *In vitro* study for the assessment of poly(L-Aspartic acid) as a drug carrier for colon-specific drug delivery. *Int. J. Pharm.* **126**:139–145 (1995).
16. C. S. Leopold and D. R. Friend. *In vivo* pharmacokinetics study for the assessment of poly(L-Aspartic acid) as a drug carrier for colon specific drug delivery. *J. Pharmacokinet. Biopharm.* **23**: 397–406 (1995).
17. K. Kobashi, T. Nishimura, M. Kusaka, M. Hattori, and T. Namba. Metabolism of sennosides by human intestinal bacteria. *Planta Medica.* (*J. Med. Plant Res.*) **40**:225–236 (1980).
18. H. J. Pownall, D. L. Hickson, and L. C. Smith. Transport of Biological Lipophills: Effect of lipophilic structure. *J. Am. Chem. Soc.* **105**:2440–2445 (1983).
19. J. Conchie and D. C. MacDonald. Glycosidase in the mammalian alimentary canal. *Nature* **184**:1233 (1959).
20. A. Rubinstein. Approaches and opportunities in colon-specific drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **12**:101–149 (1995).
21. D. R. Friend and G. W. Chang. Drug glycosides: Potential prodrugs for colon-specific drug delivery. *J. Med. Chem.* **28**:51–57 (1985).
22. D. R. Friend and T. N. Tozer. Drug glycosides in oral colon-specific drug delivery. *J. Control. Release* **19**:109–120 (1992).
23. D. R. Friend, S. Phillips, A. McLeod, and T. N. Tozer. Relative antiinflammatory effect of oral dexamethasone-β-D-glucoside and dexamethasone in experimental inflammatory bowel disease in guinea pig. *J. Pharm. Pharmacol.* **43**:353–355 (1991).
24. R. R. Scheline. Drug metabolism by intestinal microorganism. *J. Pharm. Sci.* **57**:2021–2037 (1968).
25. J. W. Simpkins, M. Smulkowski, R. Dixon, and R. Tuttle. Evidence for the delivery of narcotic antagonists to the colon as their glucuronide conjugates. *J. Pharmacol. Exp. Ther.* **244**:195–205 (1988).
26. B. Haeberlin, W. Rubas, H. W. Nolen, III, and D. R. Friend. *In vitro* evaluation of dexamethasone-β-D-glucuronide for colon-specific drug delivery. *Pharm. Res.* **10**:1553–1562 (1993).
27. N. Cui, D. R. Friend, and R. N. Fedorak. A budesonide prodrug accelerates treatment of colitis in rats. *Gut* **35**:1439–1446 (1994).
28. H. W. Nolen, III, R. N. Fedorak, and D. R. Friend. Steady-state pharmacokinetics of corticosteroid delivery from glucuronide prodrugs in normal and colitic rats. *Biopharm. Drug Dispos.* **18**: 681–695 (1997).
29. H. W. Nolen, III and D. R. Friend. Menthol-β-D-glucuronide: A potential prodrug for treatment of the irritable bowel syndrome. *Pharm Res.* **11**:1707–1711 (1994).
30. C. M. P. Rodrigues, B. T. Kren, C. J. Steer, and K. D. R. Setchell. The site specific delivery of ursodeoxycholic acid to the rat colon by sulfate conjugation. *Gastroenterology* **109**:1835–1844 (1995).
31. J. Kopecek and P. Kopeckova. N-(2-hydroxypropyl)methacrylamide copolymers for colon-specific drug delivery. In D. R. Friend (ed.), *Oral Colon Specific Drug Delivery*, CRC Press, London, 1992 pp. 189.
32. G. V. D. Mooter, B. Maris, C. Samyn, P. Augustijns, and R. Kinget. Use of azo polymers for colon-specific drug delivery. *J. Pharm. Sci.* **86**:1321–1327 (1997).
33. U. Klotz. Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-Aminosalicylic acid. *Clin. Pharmacokinet.* **10**:285–302 (1985).
34. S. P. Travis, C. Tysk, H. J. de Silva, H. Sandberg-Gertzen, D. P. Jewell, and G. Jarnerot. Optimum dose of olsalazine for maintaining remission in ulcerative colitis. *Gut* **35**:1282–1286 (1994).
35. J. P. Brown, G. V. McGarraugh, T. M. Parkinson, R. E. Wingard, Jr., and A. B. Onderdonk. A Polymeric drug for treatment of inflammatory bowel disease. *J. Med. Chem.* **26**:1300–1307 (1983).
36. M. Garretto, R. H. Riddel, and C. S. Winans. Treatment of chronic ulcerative colitis with poly-ASA: A new non-absorbable carrier for release of 5-aminosalicylate in the colon. *Gastroenterology* **84**:1162 (1983).
37. B. A. Lashner, P. M. Savina, M. Garetto, A. N. Kotale, M. B. Bushby, M. R. Blum, and C. S. Winans. Release of 5-aminosalicyclic acid (5-ASA) from BW73Y: *In vitro* and *in vivo* studies in humans. *Gastroenterology* **86**:1154–1162 (1985).
38. J. Kopecek. The potential of water-soluble polymeric carriers in targeted and site-specific drug delivery. *J. Control. Release* **11**: 279–290 (1990).
39. J. Kopecek, P. Kopeckova, H. Brondsted, R. Rathi, B. Rihova, P.-Y. Yeh, and K. Ikesue. Polymers for colon-specific drug delivery. *J. Control. Rel.* **19**:121–130 (1992).
40. G. V. Mooter, C. Samjn, and R. Kinget. The relation between swelling properties and enzymatic degradation of azo-polymers. *Pharm. Res.* **11**:1737–1741 (1994).
41. S. Davaran, J. Hanaee, and A. Khosravi. Release of 5-amino salicylic acid from acrylic type polymeric prodrugs designed for colon specific drug delivery. *J. Control. Release* **58**:279–287 (1999).
42. B. Flourie, C. Molis, L. Achour, H. Dupas, C. Hatat, and J. C. Rambaud. Fate of β-cyclodextrins in the human intestine. *J. Nutr.* **123**:676–680 (1993).
43. A. Gerloczy, A. Fonagy, P. Keresztes, L. Perlaky, and J. Szejtli. Absorption, distribution, excretion and metabolism of orally administered ^{14}C-β-cylodextrin in rat. *Arzneim. Forsch. Drug. Res.* **35**:1042–1047 (1985).
44. F. Hirayama, K. Minami, and K. Uekama. *In vitro* evaluation of Biphenylyl acetic acid-β-cyclodextrin conjugates as colon-targeting prodrugs: Drug Release Behaviour in rat biological media. *J. Pharm. Pharmacol.* **48**:27–31 (1996).
45. E. Hultin and L. Nordstrom. Investigations on dextranase 1. On the occurrence and the assay of dextranase. *Acta Chem. Scand.* **3**:1405–1417 (1949).
46. T. W. Sery and E. J. Hehre. Degradation of dextrans by enzymes of intestinal bacteria. *J. Bacteriol.* **71**:373–380 (1956).
47. E. L. Rosenfeld and I. S. Lukomskaya. The splitting of dextran and isomaltose by animal tissues. *Clin. Chim. Acta* **2**:105–113 (1957).
48. E. J. Hehre and T. W. Sery. Dextran-splitting anaerobic bacteria from the human intestine. *J. Bacteriol.* **63**:424–426 (1952).
49. M. Inoue, M. Morikawa, M. Tsuboi, T. Yamada, and M. Sogiura. Hydrolysis of ester type drugs by the purified esterases from human intestinal mucosa. *Int. J. Pharm.* **29**:17–21 (1979).
50. E. Harboe, M. Johansen, and C. Larsen. Macromolecular prodrugs. VI coupling of the highly lipophilic agent naproxen to dextran and *in vitro* characterization of the conjugates. *Farmaci Sci. Ed.* **16**:73–85 (1988).
51. E. Harboe, C. Larsen, and M. Johansen. Macromolecular prodrug X. Bioavailability of naproxen after oral administration of dextran-naproxen ester conjugates in rabbits. *Farmaci Sci. Ed.* **16**:65–72 (1988).
52. E. Harboe, C. Larsen, M. Johansen, and H. P. Olesen. Macromolecular prodrug XIV. Absorption characteristics of naproxen after oral administration of dextran T-70-naproxen ester prodrug in pigs. *Int. J. Pharm.* **53**:157–165 (1989).
53. E. Harboe, C. Larsen, M. Johansen, and H. P. Olesen. Macromolecular prodrug XV. Colon-targeted delivery-bioavailability of

naproxen from orally administered dextran-naproxen ester prodrugs varying in molecular size in the pig. *Pharm. Res.* **6**:919–923 (1989).
54. A. D. McLeod, D. R. Friend, and T. N. Tozer. Synthesis and chemical stability of glucocorticoid-dextran esters: potential prodrugs for colon-specific delivery. *Int. J. Pharm.* **92**:105–114 (1993).
55. A. D. McLeod, D. R. Friend, and T. N. Tozer. Glucocorticoid-dextran conjugate as potential prodrug for colon-specific delivery: hydrolysis in rat gastrointestinal tract contents. *J. Pharm. Sci.* **83**:1284–1288 (1994).
56. E. Schacht, A. Gevaert, E. R. Kenawy, K. Molly, W. Verstraete, P. Adriaensens, R. Carleer, and J. Gelan. Polymers of colon-specific drug delivery. *J. Control. Release* **39**:327–338 (1996).
57. K. Molly, M. V. Woestyne, and W. Verstraete. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl. Microbiol. Biotechnol.* **39**:254–258 (1993).
58. Y. J. Jung, J. S. Lee, H. H. Kim, Y. T. Kim, and Y. M. Kim. Synthesis and properties of dextran-5-aminosalicyclic acid ester as a potential colon-specific prodrug of 5-aminosalicyclic acid. *Arch. Pharmacal. Res.* **21**:179–186 (1998).