Expert Opinion

What should be considered on design of a colon-specific prodrug?

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Importance of the field: Generally, a prodrug, a pharmacologically inactive derivative of an active drug, is designed to modulate pharmacokinetic properties of the parent drug. Targeted distribution of an orally administered drug at the large intestine confers therapeutic advantages on treatment of colonic diseases, peptide and protein therapy and chronotherapy.
Areas covered in this review: To achieve such distribution control in the gastrointestinal tract, the adoption of the prodrug concept gives birth to a colon-specific prodrug. The requirement for a prodrug to be colon-specific is described along with the necessary and sufficient conditions of drugs for conversion to a colon-specific prodrug. The known and previously unnoticed factors that negatively influence therapeutic activity and reproducibility of a colon-specific prodrug are presented with suggestions to minimize the negative influence.
What the reader will gain: This review provides tactics to satisfy the requirements for being colon-specific and the potential strategies to circumvent obstacles in developing an efficient colon-specific prodrug.
Take home message: On design of a colon-specific prodrug, one should take into consideration not only delivery of a drug to the target site, but also the therapeutic effectiveness there.

Keywords: colon-specific carrier, colon-specific prodrug, colonic metabolism, drug delivery, intestinal microflora, oral drug delivery, prodrug and site-specific drug delivery

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1. Introduction

Site-specific delivery of a drug is a useful strategy to improve therapeutic properties of the drug [1,2]. Unlike the conventional way of drugs traveling through the body, which usually leads to extensive distribution, site-specific delivery is aimed at a site where there is a therapeutic target [2]. This site-restricting control of drug distribution enables the therapeutic concentration of a drug to be reached at a target site while its blood concentration is kept low, which should result in an increase of the therapeutic potency as well as a decrease of side effects. For such advantages, delivery systems targeting a variety of tissues have been developed [3-11].

Colon-specific drug delivery implies transport of a drug to the large intestine without significant loss in the upper intestine on oral administration of the drug [12]. Colonic delivery is directed mainly at achieving efficient treatment of diseases that develop at the colonic site and reducing side effects by restricting systemic absorption [13,14]. Conventional dosage forms are designed to be disintegrated, dissolved and subsequently absorbed systemically in the upper intestine. The colon, where activity of proteolytic enzymes is lower than in the small intestine, could also be utilized as the site of absorption for those drugs that are unstable in the upper intestine, such as therapeutic peptides and proteins [15,16]. Colonic targeting may be

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Article highlights.

- Restriction of the systemic absorption of a drug in the upper intestine is linked to an efficient delivery of the drug to the large intestine.
- A great metabolic activity of microflora in the colon could be exploited for colon-specific activation of a prodrug.
- The metabolic activity of microflora in the colon could be variable among individuals and even in a single subject.
- All therapeutic agents that are applicable to treatment of colonic diseases, peptide and protein therapy and chronotherapy could be candidates for modification to colon-specific prodrugs.
- For a drug to become a colon-specific prodrug, an appropriate functional group is a prerequisite for conjugation with a colon-specific promoiety.
- Colonic distribution of the parent drug after oral administration of a colon-specific prodrug is relevant to an efficient therapeutic action against a colonic disease whose pathogenic lesion occurs not only in the proximal part but also in the distal part of the large intestine.
- The rate of bacterial enzyme-mediated conversion of colon-specific prodrugs to the parent drugs is one of the determinants to control regional therapeutic availability of the drugs in the large intestine.
- Colonic metabolic stability of drugs is one of the determinants to control regional therapeutic availability of the drugs in the large intestine.
- Insightful suggestions and potential strategies for design of an efficient colon-specific prodrug are provided.

This box summarises key points contained in the article.

applicable to delaying absorption to achieve a desirable time–concentration profile for the treatment of diseases such as asthma, gastric ulcer and arthritis, which often have peak symptoms at bedtime [17-19].

Two major approaches available for colon-specific drug delivery are pharmaceutical formulation and prodrug [20]. The pharmaceutical formulations for colonic delivery include coating or tableting with pH-sensitive or colonic (microbial) enzyme-degradable polymers and the design of timed-release dosage forms [21]. The prodrug approach is achieved by coupling a hydrophilic (or polymeric) and enzymatically disposable carrier to a candidate drug, which renders the coupled product (prodrug) non-absorbable in the upper intestine and cleavable to liberate the drug in the large intestine [22]. Generally, although applicable limitedly to drugs with a chemical group available for coupling with a colon-specific carrier, the prodrug approach confers a drug better colon-targetability than does the pharmaceutical approach.

2. Body

2.1 Restriction of systemic absorption

For a foreign compound ingested to reach the large intestine without significant systemic absorption in the upper intestine, the compound should be hydrophilic or large, which prevents passive transport through the intestinal epithelial cell layer [23,24]. Conjugation of a parent drug with hydrophilic small molecules such as sulfate, amino acids and sugars is a way to restrict systemic absorption of the drug in the upper intestine [25-27]. Another way is to conjugate with various natural or synthetic polymers. Some polymers used for this purpose, such as cyclodextrin, dextran, poly(L-aspartic acid), have biodegradable backbones susceptible to colonic bacterial enzymes [28-30]. Others, such as sulfaniamidoethylene polymer, N-(hydroxypropyl)methacrylamide copolymers and polyamidoamine dendrimer, are not degradable [31-33]. In addition, the linkage between the drug and the promoiety should be chemically and enzymatically stable during the transit through the stomach and small intestine.

2.2 Colon-specific activation of a prodrug

The intestinal microflora has a great metabolic activity participating in the metabolism of exogenous and endogenous compounds, which has a metabolic potential equal to or greater than that of the liver [34]. The number of bacteria in stomach and proximal small intestine (10^2 – 10^4 cfu/ml) is similar and increases to 105 – 107 cfu/ml in the lower part of the small intestine. In the colon, the bacterial count reaches 1011 – 1012 cfu/ml, where the anaerobic bacteria outnumber aerobes by a factor of 102 – 104 [35]. The 'atmospheric' condition in the colon is a total lack of oxygen, rendering the oxidation–reduction potential very low, which is in the range -200 to -250 mV [35,36]. As summarized in **Table 1**, differences between metabolisms in gut flora and liver are the accessibility and the type of predominant metabolic reactions [37]. In general, oxidation and conjugation are the predominant metabolic reactions in the liver, whereas reduction and hydrolysis are the preferred ones in the large intestinal lumen. This unique environment of the colon could be exploited for colon-specific activation of a prodrug, which is a pharmacologically inactive version of a parent drug. Typical drug metabolizing enzymes in human intestinal microflora, some of which have been utilized for colon-specific activation of prodrugs, are listed in **Table 2** [37]. Several reviews deal with colonic drug delivery using the prodrug strategy, in which prodrugs are introduced as being classified by promoieties or bacterial enzymes [20,22,38].

2.3 Variation in microflora

Microbially triggered activation of colon-specific prodrugs seems to be a solid strategy for the release of parent drugs from the prodrugs at the target site. However, there are some issues that should be considered for the therapeutic feasibility of the prodrugs. Inter-individual difference in microflora is one of the issues. Considerable variations in the composition of the microflora are found among individuals [35], which might result in variation in therapeutic activity of a colon-specific prodrug. In a single healthy subject, intestinal flora is quite stable over a prolonged period of time [35]; but, even in a single subject, disease states are reported to influence intestinal

Table 1. Differences between metabolism in colonic microflora and liver.

	Colonic flora	Liver
Accessibility	Limited*	All drugs systemically available
Phase I metabolism		
Oxidation	Absent	Predominant
Reduction	Predominant	Unimportant
Hydrolysis	Important (more)	Important
Phase II metabolism		
	Cleavage of Conjugation	Conjugation

*Cases for a drug to reach colonic microflora: poor intestinal absorption, biliary excretion and colon-specific delivery.

Table 2. Drug-metabolizing enzymes in the human colonic microflora.

Enzyme	Metabolic reaction catalyzed
Hydrolytic reaction	
Esterase and amidase	Cleavage of esters or amides of carboxylic acids
Glycosidase	Cleavage of β-glycosides of alcohols and phenols
Glucuronidase	Cleavage of β-glycosides of alcohols and phenols
Sulfatase	Cleavage of O-sulfates and sulfamates
Reductive reaction	
Nitroreductase	Reduction of aromatic and heterocyclic nitro compounds
Azoreductase	Reductive cleavage of azo compounds
N-oxide reductase	Removal of oxygen from N-oxide
Sulfoxide reductase	Removal of oxygen from sulfoxides
Hydrogenase	Reduction of carbonyl groups and aliphatic double bonds

flora [35,39]. Crohn's disease patients show increased *Escherichia coli* and lactobacilli in the large intestine whereas ulcerative colitis patients do not [40]. Indeed, a decrease in glycosidase and azoreductase activity is observed in the feces of patients with active Crohn's disease [41]. The disease states accompanying diarrhea, such as cholera, tropical sprue and irritable bowel disease, tend to increase aerobic bacteria or decrease anaerobic bacteria concentration in the fecal flora [35,42].

2.4 Candidate drugs for modification to colon-specific prodrugs

As described earlier, efficient treatment of colonic diseases including inflammatory bowel disease (IBD), systemic absorption of therapeutic agents that are not stable in the upper intestine, or modulation of time-concentration to match time-symptoms for chronotherapy are the cases appropriate for colon-specific prodrug approaches [18]. All drugs that are medicated to colonic diseases, IBD, constipation/diarrhea, colorectal cancer, irritable bowel disease and intestinal infection could be candidates for modification to colon-specific prodrugs, which should increase the potency and decrease the systemic side effects. Anti-inflammatory drugs, 5-aminosalicylic acid (5-ASA) and glucocorticoids, which are used commonly for treatment of IBD [43], were subjected to conjugation with hydrophilic small molecules or natural and synthetic polymers to be delivered to the large intestine [22]. In addition, colon-specific prodrugs of metronidazole, nalidixic acid and sulfa drugs (antimicrobial agents), menthol (antispastic agent), naloxone and nalmefene (anti-constipation), ursodeoxycholic acid (chemopreventive agent), 9-aminocampothecin and 5-fluorouracil (anticancer agents) were reported [32,44-50]. Naturally occurring glycosides, rutin and sennosides, act as colon-specific prodrugs that deliver to the large intestine and liberate their aglycons, quercetin and rhein anthrone, followed by exerting their respective biological activities, anti-inflammatory and laxative effects [51,52]. Besides the above colon-specific prodrugs for treatment of colonic diseases, immunosuppressive agents (treatment of IBD), vermicides (treatment of intestinal parasites) and COX-2 selective inhibitors (prevention of colorectal cancer) are candidate drugs for modification to colon-specific produgs. In fact, colonic delivery of mebendazole, a vermicide, and celecoxib, a selective COX-2 inhibitor, using pharmaceutical formulations has been reported [53-55]. No report has been published on a prodrug for colonic delivery of peptide and protein drugs, while vasopressin, insulin and calcitonin were pharmaceutically formulated to be delivered to the large intestine for systemic absorption [16,56,57]. A capsule or pellet containing peptide therapeutics was coated with synthetic polymers carrying an azo bond in the backbones, which are cleavable by bacterial azoreductase in the colon [58]. Harboe *et al.* investigated pharmacokinetics of various dextran-naproxen ester conjugates administered orally in animals and found that the naproxen plasma profiles for all the polymeric prodrugs showed a characteristic lag time of naproxen appearance in the blood (2 – 3 h) compared with the administration of the parent drug [29]. Similar delayed absorption of ketoprofen was observed in the study on various dextran-ketoprofen ester conjugates [59]. The authors reasoned that the delay in systemic absorption is due to colonic delivery of the dextran conjugates and subsequent release of the parent drugs from the conjugates. The delayed systemic absorption may be advantageous to the management of symptoms of arthritis, asthma and gastric ulcer, which may commonly have a peak symptom at bedtime. Therefore, therapeutic agents for the treatment of such diseases should also be potential candidate drugs for modification to colon-specific prodrugs. These include non-steroidal anti-inflammatory agents,

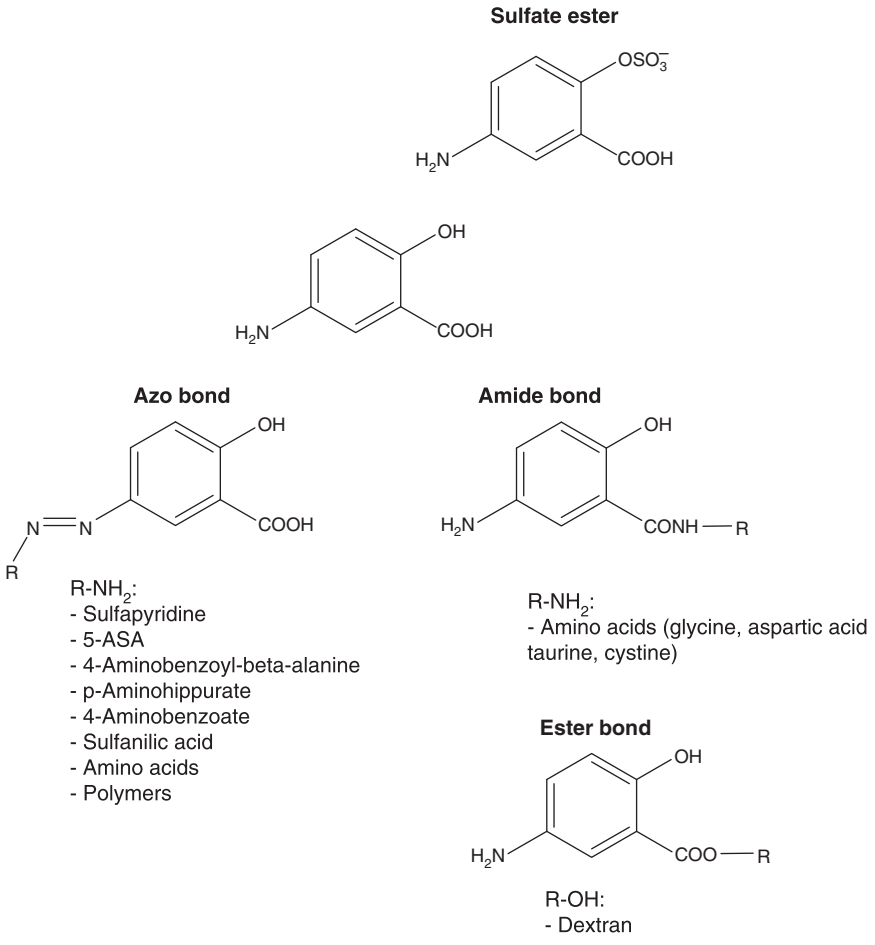

Figure 1. Various chemical modifications imposed on 5-aminosalicylic acid to couple colon-specific promoieties to 5-aminosalicylic acid.

histamine receptor 2 blockers, proton pump inhibitors and antiasthmatic agents.

2.5 Functional groups appropriate for coupling with a colon-specific promoiety

Candidate drugs have been introduced above, which should become more efficient in therapeutic aspects when modified to a colon-specific prodrug. Unfortunately, not all the candidate drugs can be modified to such a prodrug. For a drug to become a colon-specific prodrug, an appropriate functional group is a prerequisite for conjugation with a colon-specific promoiety, where the covalent link should be stable in the upper intestine and cleavable in the large intestine.

5-Aminosalicylic acid, an anti-inflammatory agent, is the mainstay for inducing and maintaining remission in mild-to-moderate IBD such as ulcerative colitis and Crohn's disease [43]. As shown in **Figure 1**, various chemical modifications have been imposed to 5-ASA to deliver the drug specifically to the target site [22]. Three functional groups in 5-ASA, aromatic amine, carboxylic group and phenol, have been utilized for conjugation with a promoiety. The aromatic amine was conjugated with sulfapyridine, another 5-ASA, 4-aminobenzoyl-β-alanine, *p*-aminohippurate, 4-aminobenzoate, sulfanilic acid and amino acids via an azo bond, namely, sulfasalazine, olsalazine, balsalazide, ipsalazide, bensalazide, salicylazosulfanilic acid and 5-ASA-amino acid azo conjugates, respectively [60-63]. Polymers with a pendant group that is able to form an azo bond on reaction with 5-ASA were reported as polymeric colon-specific prodrugs of 5-ASA [31,33,64]. Recently, 5-ASA was conjugated with diamino-poly(ethylene glycol) by means of an azo bond for colon-specific delivery [65]. The physicochemical nature of the polymeric 5-ASA prodrugs depends on monomers consisting of the polymers, which affects enzymatic release of 5-ASA [66]. 5-ASA derivatives where the amine was conjugated with the carboxylic group in dipeptides were developed. However, they were designed to release 5-ASA in the small intestine [67].The carboxylic group in 5-ASA was coupled with amino acids by means of an amide bond, which is susceptible to colonic bacterial amidase but not to host ones in the upper intestine [26]. In the case of using amino acids as a promoiety, some amino acids such as taurine, cysteine and phenylalanine [63,68,69] play dual roles, not only

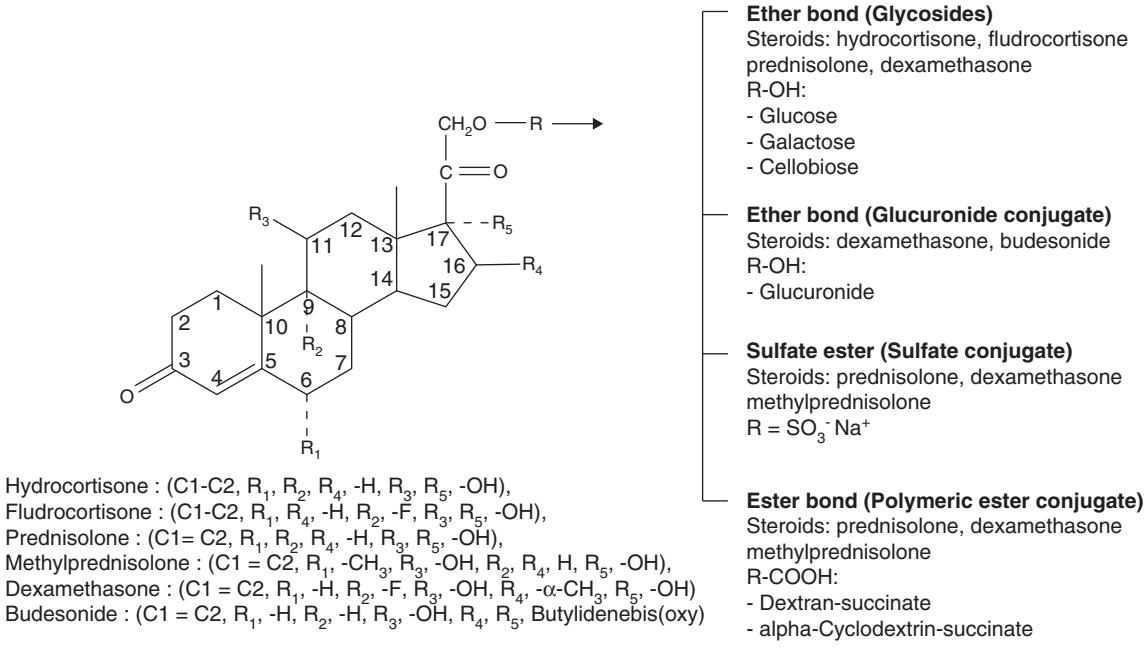

Figure 2. Various chemical modifications imposed on glucocorticoids to couple colon-specific promoieties to the glucocorticoids.

delivering 5-ASA to the large intestine but also enhancing the therapeutic effects of 5-ASA on experimental colitis. Kim *et al.* revealed a molecular mechanism for the taurine effect, which an additive inhibitory effect of 5-ASA and taurine on the inflammatory transcription factor, NF-κB, was involved in [70]. The carboxylic acid was conjugated with dextran by means of an ester bond [71]. Although an ester bond is not a linkage suitable for colon-specific delivery considering that esterases are abundant in the whole gastrointestinal (GI) tract without significant regional preference [72], dextran-drug ester conjugates including dextran-5-ASA ester conjugates show a good colon-targetability. Larsen *et al.* suggest that the ester bond between drugs and the polymeric carrier dextran is protected by steric hindrance from esterase attack in the upper intestine, whereas the ester bond is exposed by degradation of the dextran backbone to oligomers by bacterial dextranases in the colon, resulting in hydrolysis and liberation of parent drugs [73]. The phenol group in 5-ASA was also used for coupling with a promoiety. The phenol was sulfated to afford 5-ASA sulfate ester, which should be more hydrophilic than 5-ASA and is desulfated in the colonic contents while stable in the contents of the upper intestine, thereby satisfying the requirement for colon targeting [74].

Glucocorticoids, which have been used for attack therapy against IBD, are well absorbed in the upper intestine, causing serious side effects on long-term administration [75]. Thus, development of a colon-specific delivery system for a glucocorticoid is highly desirable. The hydroxyl groups in glucocorticoids are the sites for coupling with a promoiety, as shown in **Figure 2**. Sugars such as glucose, galactose and cellobiose were attached to the hydroxyl groups of glucocorticoids to afford glycosides [27,76]. The glycosidic ether linkages between sugars and glucocorticoids are cleaved to release the active drugs by bacterial glycosidases in the colon, whose release rate depends on sugars [76]. Luminal glucosidase activity is present in the small intestine, which is from sloughed mucosa cells in the GI tract [77]. However, glycoside prodrugs do not seem to be degraded to a significant extent in the small intestine because the glucosidase activity is lower than that in the colon [76]. Dexamethasone and budesonide-glucuronide conjugates were introduced as colon-specific prodrugs, where the hydroxyl groups of the steroids were modified to glucuronide ether linkages [78,79]. As luminal β-D-glucuronidase activity has greater regional preference to the colon than luminal β-D-glucosidase activity, the glucuronide prodrugs are suggested to have better colonic delivery efficiency than glucoside prodrugs [78]. Doh and co-workers added sulfate ester conjugation to modifications of glucocorticoids for colon-specific prodrugs [25,80]. Sulfated metabolites excreted through the bile duct are known to be desulfated by bacterial sulfatases in the large intestine. This colonic metabolism was taken advantage of to deliver a chemopreventive agent, ursodeoxycholic acid (UDCA), to the colon. Rodrigues *et al.* reported that sulfated UDCA, which is greatly hydrophilic, was delivered specifically to the large intestine without significant loss in the upper intestine compared with UDCA [49]. In the same way, sulfation of glucocorticoids renders the parent drugs more hydrophilic, limiting their systemic

absorption, which is linked to efficient delivery to the large intestine [81]. Side effects including adrenal suppression are attenuated significantly comparing the sulfate prodrugs with the parent steroids, whereas the potency of the prodrugs is greater than that of the corresponding steroids [82,83]. Dextran is also used as a colon-specific promoiety for glucocorticoids. The hydroxyl groups in the drugs and the carrier are conjugated by means of a linker molecule, succinic acid, where esters are formed at both ends of the linker molecule. The glucocorticoid-succinate-dextran conjugates act as a polymeric colon-specific prodrug and show fewer side effects and greater potency than the corresponding steroids [84,85]. Like dextran-succinate-glucocorticoid conjugates, α-cyclodextrin-succinate-prednisolone conjugate, where α-cyclodextrin was used as a carrier for colon-specific delivery of the steroid, possesses similar advantages over the parent drug [86,87].

5-Fluorouracil (5-FU), used for a variety of cancers including colorectal cancer, is administered parenterally because oral administration of the drug shows low and variable bioavailability owing to the incomplete absorption and marked bioinactivation by dihydrouracil dehydrogenase in the liver and the mucosal membrane of the GI tract [88]. Bioactivation of 5-FU to 5-fluoro-2′-deoxyuridine was greater in colon cancer than in normal tissue whereas bioinactivation is minimal in the colon and colon tumor, suggesting that colon-specific delivery of 5-FU should improve the therapeutic property of 5-FU [89,90]. The nitrogens in the pyrimidine ring of 5-FU might be available for secondary amide formation but not for diazo reaction. Thus, amino acid conjugation by means of an amide bond may be a feasible way to design a colon-specific prodrug of 5-FU, which remains to verify whether the secondary amide acts as a colon-specific linkage and the steric hindrance does not prevent enzymatic activation. To avoid such uncertainty, Lee *et al.* designed *N*-Acyl-2-acetoxy-DL-glycine (NAG) as a chemical drug delivery system for colonic delivery of 5-FU (**Figure 3A**) [50]. The pyrimidine nitrogen (N-1 position) in 5-FU was substituted with the acetoxy group to afford *N*-nicotinoyl-2-(5-fuorouracil-1-yl)-DL-glycine (NFG), in which the nicotinoyl amide is proposed to be cleaved by bacterial enzymes, immediately followed by spontaneous degradation resulting in release of 5-FU in the large intestine, as shown in **Figure 3B**. It was found that the release per cent of 5-FU in the cecal contents is low (9% of the dose), probably owing to the steric hindrance imposed by 5-FU against enzymatic cleavage of the amide bond. Another glycyl moiety was inserted next to the nicotinoyl group of NFG to decrease the steric hindrance, expecting enhanced hydrolysis of nicotinoyl group to release 5-FU more readily than NFG. [91]. The 5-FU prodrug (**Figure 3C**) liberated 5-FU in the cecal contents, which increased 5-FU release up to 16% of the dose. The colon-specific chemical delivery system, NAG, was applied to colon-specific delivery of an amebicidal agent, metronidazole (MTZ) [44]. The hydroxyl group in MTZ was easily substituted with the acetoxy group to produce a colon-specific prodrug of MTZ, *N*-nicotinoyl-2-{2-(2-methyl-5-nitroimidazole-1-yl) ethyloxy}glycine (**Figure 3D**), whose release mechanism is thought to be the same as that of the 5-FU prodrug. In *in vitro* and *in vivo* experiments, the MTZ prodrug was shown to be delivered to and released MTZ specifically at the large intestine. The authors suggest that the colon-specific chemical delivery system should be applicable to any drugs with an appropriate nucleophile that is able to be substituted with the acetoxy group.

2.6 Colonic distribution of the parent drugs after oral administration of colon-specific prodrugs

For an efficient therapeutic action against a colonic disease such as inflammatory bowel disease, a colon-specific prodrug is required to deliver an active agent not only to the proximal part but also to the distal part of the large intestine where inflammatory lesion of target diseases frequently occurs [14]. In this respect, the colonic metabolic susceptibility of both a parent drug and its prodrug should be considered on design of a colon-specific prodrug. If the prodrug is very susceptible to colonic metabolism(s), almost all of it would be converted to the parent drug at the proximal part of the large intestine and would not reach the distal large intestine. In this case, it might depend on the colonic metabolic stability of the parent drug whether the drug could reach the distal large intestine. If the prodrug has an appropriate half-life for the metabolic conversion, substantial amount of the prodrug would arrive at and be converted to the active agent in the distal large intestine, thus probably affording the active agent regardless of the metabolic stability of the active agent. Of course, the more stable the active agent, the more it would be accumulated at the distal large intestine. Therefore, it is thought to be important to select an active agent and a colon-specific promoiety that have a proper metabolic property for design of an efficient colon-specific prodrug. The following two sections deal with more details on these issues.

2.6.1 Efficiency of bacterial enzyme-mediated activation of a prodrug

This issue involves the rate of bacterial enzyme-mediated conversion of colon-specific prodrugs to the parent drugs, which would affect regional therapeutic availability of the parent drugs in the large intestine after oral administration of colon-specific prodrugs. The rate of a colon-specific prodrug conversion to a parent drug depends mainly on susceptibility of the drug-promoiety linkages to colonic metabolisms. Even in the case of the same linkage, the susceptibility should vary depending on promoieties and the parent drugs. The conversion rates of 5-ASA-amino acid conjugates were compared varying the promoiety, amino acids. The order of the conversion rates is 5-ASA-glycine > 5-ASA-taurine > 5-ASA-aspartic acid in the cecal contents [92], which is in a parallel pattern with fecal recovery of 5-ASA and the prodrugs [68]. The fecal recovery of the prodrugs increases as the *in vitro*

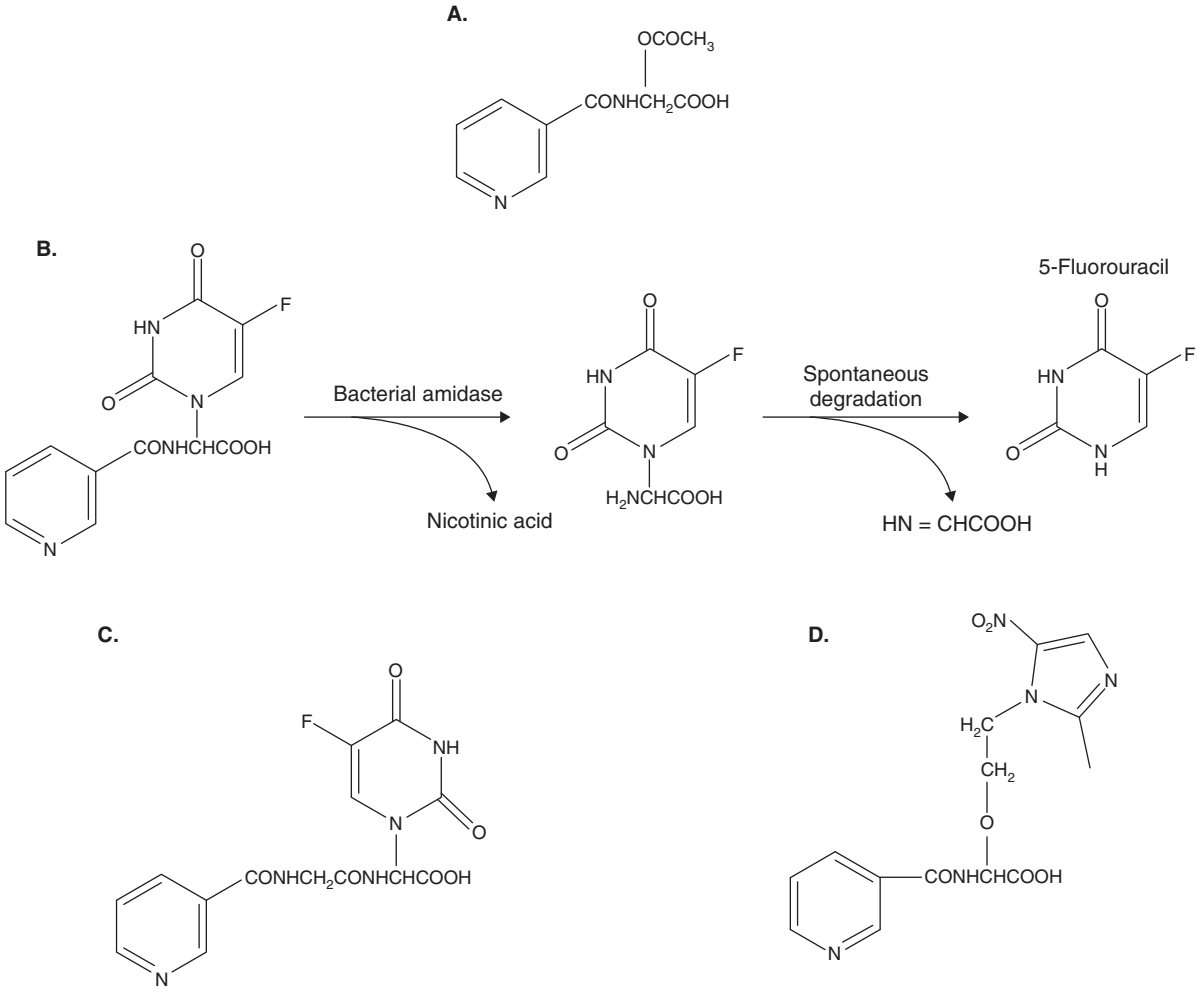

Figure 3. A colon specific chemical drug delivery system of 5-fluorouracil and metronidazole. A. *N*-nicotinoyl-2-acetoxy-D, L-glycine, a chemical drug delivery system for colonic delivery of drugs [50]. **B.** Proposed pathway for the bioactivation of *N*-nicotinoyl-2-(5-fuorouracil-1-yl)-D,L-glycine, a colon-specific prodrug of 5-fluorouracil [50]. **C.** *N*-nicotinoylglycyl-2-(5-fluorouracil-1-yl)-D,L-glycine, a colon-specific prodrug of 5-fluorouracil with another glycine between nicotinic acid and glycine of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D, L-glycine [91] **D.** *N*-Nicotinoyl-2-{2-(2-methyl-5-nitroimidazole-1-yl)ethyloxy}glycine, a colon specific prodrug of metronidazole [44].

conversion rate becomes low. On the other hand, glucocorticoid sulfate ester conjugates were deconjugated at varying rates depending on the parent drugs. The order was prednisolone sulfate (PDS) > methylprednisolone sulfate (MPS) > dexamethasone sulfate (DS) in the cecal contents [81]. An *in vivo* study carried out with MPS and DS demonstrated no fecal recovery of the steroid prodrugs after oral administration [82,83]. However, it is not certain whether the difference in the extent of the prodrug conversion originates from the difference in conversion rates or the size of the dose, considering that oral doses of 5-ASA prodrugs are ~ 5 – 12 times those of steroid prodrugs under the assumption that a much longer time should be taken for complete conversion at larger doses [68,82,83].

For prodrugs with an azo bond, the electron density of the azo region affects the rate of the reductive bond cleavage. A low electron density within the azo region facilitates reduction to form amine via the hydrazo intermediate. Thus, introduction of an electron-withdrawing group into an aromatic ring linked to an azo bond accelerates the azo bond cleavage [93].

The drug release from polymeric colon-specific prodrugs such as dextran-drug conjugates is initiated by the depolymerization of the polymer backbone, which determines the rate of drug release [73]. The dextranase-mediated depolymerization of dextran-5-ASA ester conjugates was attenuated compared with that of unsubstituted dextran, which depends on the degree of substitution and hydrophobicity of a drug coupled to dextran [71]. Accordingly, the candidate drug for the polymeric prodrug should either be hydrophilic or have high efficacy in order to provide effective dose on administration of a practical dose of prodrug. In other words, for the drug

with high efficacy (small dose), an effective dose might be achieved successively from a polymer drug with low degree of substitution. If the drug has low efficacy (large dose) the degree of substitution of the polymer drug should be high so that the effective dose will be provided from the polymer drug. In this case, the drug should be hydrophilic enough for facile bioconversion of the polymer drug.

In an *in vitro* experiment carried out with dextran-drug ester conjugates (degree of substitution: 20), treatment with dextranase for 8 h depolymerized the dextran-5-ASA ester conjugate up to 70%, whereas the dextran ester conjugates of flufenamic acid and 4-(4-ethoxycarbonylphenylazo)salicylic acid, which are more lipophilic than 5-ASA, were depolymerized up to 11 and 7.4%, respectively (unpublished data). Utilization of dextran as a colon-specific carrier seems to be suitable for hydrophilic drugs or for drugs whose effective doses are not high if they are hydrophobic.

In polymeric prodrugs with chemically and enzymatically stable backbones holding an azo bond in the pendant group as a colon-specific linkage, it seems that azo bonds are still cleavable because the reductive cleavage possibly takes place outside the bacterial cells by the small molecular reducing agents such as FMN and NADP, which are accessible to the azo bonds without the backbone degradation, functioning as a shuttle for the electron transport [31,94].

2.6.2 *Metabolic stability of a parent drug in the colon*

Metabolic stability of not only a colon-specific prodrug but also a parent drug could influence the regional therapeutic availability in the large intestine. The level of the parent drug at the target site should result from the net accumulation resulting from relative metabolic stability of a parent drug and its prodrug. The level of the drug depends mainly on the conversion rate of the prodrug if the parent drug is metabolically stable. In the other case, the drug should be at a certain level, which is determined by the difference between the rates of the prodrug conversion and the parent drug metabolism in the large intestine. Glucocorticoids are subject to reductive metabolism in the large intestine, which inactivates the drugs [95]. Kong *et al.* compared the relative metabolic stability of glucocorticoids and their sulfate prodrugs in the rat cecal contents and observed different accumulation profiles of drugs released from the prodrugs [81]. The accumulation of methylprednisolone and prednisolone, which are vulnerable to reductive metabolism, was lower than that of dexamethasone, which is resistant to it, in the rat cecal contents. The accumulation profiles (as a function of time) represented the net amounts of the steroids produced from corresponding prodrugs and disappeared metabolically. Consistent with this observation, in the large intestine the maximal concentration of dexamethasone after oral administration of DS (dose: 1 mg/kg) to rats was ~ 43 μg/ml, whereas that of methylprednisolone after MPS administration (dose: 2.36 mg/kg) was 45 μg/ml and the total amounts of dexamethasone and methylprednisolone accumulated for 8 h in the large intestine were 630 and 250 μg, respectively [82,83]. Vulnerability of various glucocorticoids to reductive metabolism in the rat cecal contents was investigated [81,96]. Friend and Chang reported that the dexamethasone recovery in the cecum is much greater than that of prednisolone 4 – 5 h after oral administration of glucosides of prednisolone and dexamethasone, which is because of the difference in delivery efficiency of the two prodrugs [27]. However, the different susceptibility of glucocorticoids to the reductive metabolism suggests that the lower recovery of prednisolone comes from metabolic instability rather than delivery efficiency. A similar phenomenon was observed when rutin, a glycoside of quercetin, was incubated in the rat cecal contents. The natural product was deconjugated to liberate quercetin, which was metabolized by bacterial enzymes. The accumulation level of quercetin was much lower than for quercetin produced from rutin [51].

Metronidazole, an anti-amebic agent, is susceptible to reductive metabolism, which is relevant to bioactivation. Incubation of MTZ alone with the cecal contents resulted in rapid disappearance, indicating extensive metabolism of MTZ, which explains why MTZ was detected at a very low level compared with the disappearance of *N*-nicotinoyl-2-{2-(2-methyl-5-nitroimidazole-1-yl)ethyloxy}glycine, a colon-specific MTZ prodrug, in the cecal contents on *in vitro* incubation or oral administration [44]. The authors suggest that a large fraction of MTZ produced from the prodrug was reduced to the active metabolite in the colonic bacteria, thus the observation that MTZ remained at a low level in the large intestine presents evidence that the prodrug should elicit effective antimicrobial activity.

Basit *et al.* reported that histamine receptor 2 blockers, a candidate drug for modification to a colon-specific prodrug, are metabolized rapidly by colonic bacteria. Ranitidine and nazatidine but not cimetidine and famotidine are susceptible to the colonic metabolism [97]. Other than the aforementioned drugs, 5-ASA, nalidixic acid, naproxen and ketoprofen do not seem to be metabolized significantly in the cecal contents. *N*-acetylation of 5-ASA was not detected on incubation with the cecal contents, whereas it was detected at a significant level in the colonic contents and the blood after oral administration of colon-specific 5-ASA prodrugs or 5-ASA [26,98,99], strongly suggesting 5-ASA metabolism in the colonic tissue and the liver. As it should affect therapeutic property of their colon-specific prodrugs, colonic metabolic stability of parent drugs needs to be taken into account for selecting a candidate drug on modification to a colon-specific prodrug.

3. Conclusion

This article has reviewed what should be considered on designing a colon-specific drug delivery system using prodrug approaches. As mentioned, the requirement for colonic delivery of a drug can be satisfied by various chemical modifications of the drug, which make it hydrophilic or large enough to prevent systemic absorption in the upper intestine, and can be

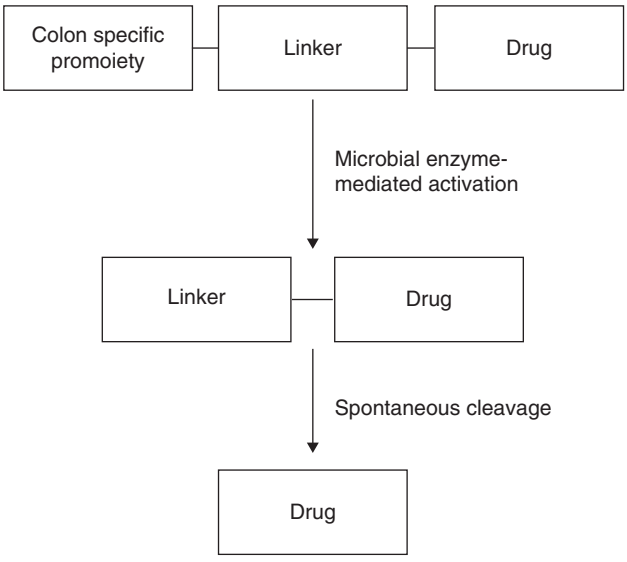

Figure 4. General principle of the bioactivation of a colon-specific chemical drug delivery system.

restored to the intact drug by microbial enzymes in the large intestine but not by host ones in the upper intestine. Prodrug approaches are a very efficient way to deliver a drug to the last part of the GI tract, which provides therapeutic advantages for treatment of colonic diseases, protein and peptide therapy and chronotherapy. However, many obstacles should be overcome to develop a clinically useful colon-specific prodrug. Chemical modification of a drug entails safety issues and requires an appropriate functional group that may not exist in all candidate drugs. In addition, a difference in colonic microflora among individuals and even in a single subject is likely to result in variation in therapeutic effects of colon-specific prodrugs. Colonic distribution of the parent drug after oral administration of a colon-specific prodrug is relevant to an efficient therapeutic action against a colonic disease whose pathogenic lesion occurs not only in the proximal part but also in the distal part of the large intestine. The regional therapeutic availability at the target site depends on colonic metabolic stability of the prodrug and the parent drug. On design of a colon-specific prodrug, the factors that influence therapeutic activity and reproducibility should be taken into consideration, which could be controlled by various tactics that have been developed and extensive knowledge that has been accumulated in the field of colon-specific drug delivery using prodrug approaches.

4. Expert opinion

The term prodrug was first introduced by Albert in 1958 to describe compounds that must undergo a chemical transformation within the body to elicit therapeutic action. However, it was not until the early 1970s that worldwide attention was directed to this area as a potential chemical approach to improving drug therapy [100]. Over the past 30 years since then, this technique has undergone considerable expansion, largely as a result of the increased awareness and understanding of the physicochemical and biological factors that determine the efficacy of prodrugs, which has led the role of prodrugs to increase in modern drug discovery programs. Of the total of 49 new chemical entities approved in the United States in 2001 and 2002, prodrugs occupied 22 – 24% of the new drugs. A recent analysis of a listing of all drugs approved worldwide estimated that 5 – 7% were in fact prodrugs. Considering that the number of prodrug patents and papers searched in PubMed and SciFinder each year has increased significantly over the last 10 years (1993 – 2003), it is likely that the portion of prodrugs in the new chemical entities approved will grow in the future [101].

Prodrug approaches are a feasible way to deliver a drug specifically to the large intestine, probably leading to improved pharmacological and toxicological properties of the drug. The great specificity in delivery and the potential for patent protection can act as a driving force to developing a colon-specific prodrug. On the other hand, there are several barriers to be overcome for the development of clinically useful colon-specific prodrugs. Entailing chemical modification of a drug, prodrugs are considered to be new molecular entities, which raises issues on safety. Before reaching the market, the prodrugs must come through animal and human studies on safety [20]. The requirement for functional groups in drugs for chemical modification puts a limitation on the general application of the approach to drugs. Other than the seem-to-be-inborn disadvantages, inter- and intra-individual variation in prodrug activation and the colonic metabolism of parent drugs may add difficulty in developing a practical colon-specific prodrug. One strategy to resolve such obstacles is to have various colon-specific promoieties coupling with a functional group. Drugs for which hydroxyl group is available could be modified to glycosides, sulfate esters, dextran-drug ester conjugates and *N*-nicotinoyl-(drug-oxy)-DL-glycine [25,27,44,84]. Conversion of the colon-specific prodrugs to the drugs is carried out by different activation systems (bacterial enzymes) in the large intestine, which may provide an option minimizing variation in the prodrug activation and controlling the colonic distribution of the active drugs. Development of a new colon-specific promoiety should make a contribution not only to running over the hurdles in achieving a reproducible clinical effect, but also to lessening the limitation of the prodrug approach set by the requirement of a functional group. The functional group limitation can be categorized into two cases. The first category includes drugs with no available functional group. The second one includes drugs with functional groups able to couple with a colon-specific promoiety, where the bonds are not cleaved (effectively) by colonic bacterial enzymes owing to steric hindrance and/or no susceptibility to the enzymes. For the drugs in the first category, pharmaceutical formulation would be the first option for colonic delivery [21]. A smart strategy to circumvent

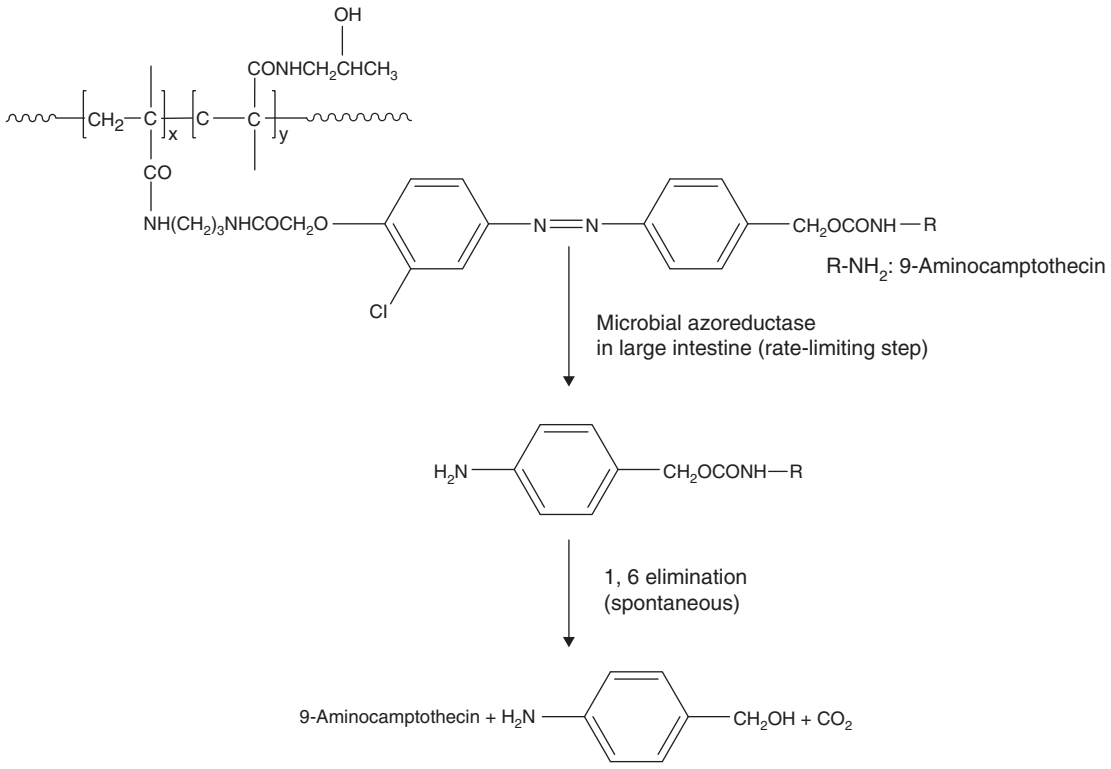

Figure 5. The bioactivation pathway of a polymeric colon specific-prodrug of 9-aminocamptothecin with azo bond and a self-1,6 eliminating linker in the pendant group [102].

the drawback derived from the second is the introduction of a chemical drug delivery system. The chemical drug delivery system generally refers to the use of pro-prodrugs, and the general principle of a colon-specific chemical drug delivery system is illustrated in **Figure 4**. The colon-specific cleavage takes place between the colon-specific promoiety and the linker to form intermediate drug-linker derivative that decomposes spontaneously to liberate the parent drug. The linker acts as a spacer to avoid steric hindrance between the linker and the promoiety and the spontaneous liberation of the parent drugs from the linker acts as a mechanism to circumvent no (efficient) enzymatic liberation of the parent drug. Lee *et al.* adopted the pro-prodrug concept to colon-specific prodrug (of 5-FU) for the first time, as shown in **Figure 3B** [50]. Gao *et al.* used a more elaborate chemical delivery system with a polymeric colon-specific prodrug, where a self-eliminating linker functioning with 1,6 elimination chemistry was used as a spacer between HPMA copolymer and 9-aminocampothecin (9-AC) and was triggered by colon-specific azo reduction, resulting in spontaneous liberation of 9-AC, as shown in **Figure 5** [102]. This polymeric prodrug demonstrated enhanced 9-AC release compared with a polymeric prodrug of 9-AC using an aromatic azo bond and dipeptide as a spacer that requires the enzymatic cleavage between 9-AC and an amino acid residue to liberate 9-AC following the reduction of the azo bond [32]. Regarding colonic metabolism of parent drugs that could result in therapeutic variation and loss of their colon-specific prodrugs, replacing such a drug with a metabolically stable one would be the first choice to resolve the problem. For example, prednisolone, which is susceptible to reductive metabolism (bioinactivation), could be substituted with metabolically stable dexamethasone on selection of a glucocorticoid as a parent drug for modification to a colon-specific prodrug. As an alternative, it could be considered to co-administer enzyme inhibitors. The possibility of this method was shown in a paper demonstrating that reduction of metronidazole was partly prevented in the presence of reduction inhibitors in the cecal contents [44]. To avoid side effects and loss in the upper intestine on co-administration of enzyme inhibitors, the inhibitors should also be colon-specific. In this sense, natural glycosides such as rutin and glycyrrhizin, which possess both colon specificity and inhibitory activity on reductase [51,103,104], could be applicable to co-administration with reductase-sensitive glucocorticoids including prednisolone for treatment of IBD. There may be advantages of co-administration over replacement with a reductase-resistant glucocorticoid (dexamethasone). As prednisolone is much more vulnerable to metabolism in the liver than dexamethasone [81], co-administration, which may stabilize prednisolone at the colon but not in the liver,

could increase therapeutic concentration at the target site but not in the blood, probably resulting in a lower possibility of systemic side effects than replacement with dexamethasone. Another advantage is that the glycoside reductase inhibitors, which have an anti-inflammatory effect on experimental colitis [51,105], may potentiate the anti-inflammatory effect of glucocorticoids against IBD on co-administration. Although delivering drugs to the last part of the GI tract, the large intestine, is not an easy task, colonic drug delivery is definitely worth all the trouble considering its therapeutic advantages. Prodrug approaches, an attractive option for colonic drug delivery, can be extended by diversifying and organizing tactics to provide colon-targetability for drugs, wherein the strategy based on chemistry would make a greater contribution to the site-specific drug delivery.

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Declaration of interest

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