Targeting Colon Drug Delivery by Natural Products

Hyunjo Kim

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http://dx.doi.org/10.5772/52346

1. Introduction

Inflammatory Bowel Disease (IBD) classified with Ulcerative Colitis (UC) and Crohn Disease (CD) is an idiopathic, life-long, destructive chronic inflammatory disease in gastrointestinal tract [1] and probably multi-factorial disease caused by interplay of the external and internal environment. Little is known about the mechanism of pathogenesis of the disease but it has been reported that immunological mechanisms are involved in etiology. Under normal situations, the intestinal mucosa is in a state of controlled inflammation regulated by a delicate balance of pro-inflammatory (tumor necrosis factor [TNF]-alpha, interferon [IFN]-gamma, interleukin [IL]-1, IL-6, IL-12 and anti-inflammatory cytokines (IL-4, IL-10, IL-11), where particularly, IL-6 stimulates T-cell and B-cell proliferation and differentiation.

Therefore, the mucosal immune system is the central effect or of intestinal inflammation and injury, with cytokines playing a central role in modulating inflammation [2,3].

During last a couple of decade, the therapeutic agents for IBD have been changed rapidly and anti-inflammatory agents such as corticosteroid and salicylates or its metabolite were used but recently biological agents are introduced. Emerging changes in IBD medications or their use for an instance, balsalazid, budesonide, 5-aminosalicylate (5-ASA) and purine analogues such as azathioprine are improvements in conventional application, additionally, mycophenolate mofetil (MMF), thalidomide and heparin are newly introduced into IBD therapy [4-6].

On the contrary, advances in molecular technology have enabled the development of novel and potentially effective targeted therapies with anti-TNF particularly infliximab, interferon-gamma and interleukin [7, 8]. Nevertheless, biologically active agents have some problems in terms of long term storage conditions and immune-toxicity, additionally biocompatibility against major histological complex (MHC) of immunoglobulin G, which may cause in inconvenience of patient compliance and more expenditure. Thus, the great in-
terest has been focused to the interplay between the adaptive and innate natural sources not only to achieve a better understanding of the immune-pathogeneses of inflammatory bowel disease but to identify targets for even more potent intervention [9-11].

Furthermore, colonic drug delivery has gained increased importance not just for the treatment of local diseases associated with the colon but also for its potential improvement related with adverse events such as ileocecal junction (ICJ) barrier and small volume capacity.

The various strategies for targeting orally administered drugs to the colon are consisted of pH dependent polymers, azo-polymers, covalent linkage of a drug with a carrier, formulation of timed released systems, drug carriers that are degraded specially by colonic bacteria and, bio-adhesive.

2. Natural products with anti-inflammatory and anti-tumor activity

In the field of pharmaceutics, the synthetic chemistry is gradually made more efficient and precise, but also gradually changing into bio-technological applications and a return to the infinitely more variable and complex chemistry of Nature.

Organisms in Nature produce secondary metabolites with the specific purpose to gain evolutionary advantages in the competition for example living space and in the search for nutrients. With an estimated numbers of more than 300 thousands species of plants and probably close to two million species of various organisms, the biodiversity of Nature remains an unparalleled reservoir of biological and chemical diversity. However, most of the biodiversity is as yet unexplored.

Most important thing is the development of strategies for selection, isolation and characterization with the objective to discover unique bioactive chemical structures with drug potential, and to reveal unknown targets, by studying the evolutionary structure–activity optimization in Nature.

In addition to the possibility to discover new drug candidates for drug development, bioactive natural projects have potential as pharmacological tools, intermediates, or templates for synthesis of drugs.

The increased uses of herbal remedies, which contain complex mixtures of natural products, need intensified scientific studies to establish efficacy and safety of these types of products as well as clinical studies.

With the increasing interest for environmental aspects, green chemistry, and a sustainable use of natural products, this renewal could have a strategic position in bridging chemistry and biology.

The correlation between the chemical structures responsible for the shown bioactivity needs to be studied to understand the observation on a molecular level using both in vivo, in vitro and in silico methods [12]. Explanatory model the interdisciplinary nature of pharmacogn-
ocy interpreted in an explanatory model presented by Larsson and co-workers [13]. In this model a clearly defined role is presented for aspects of informatics, including bio- and chemoinformatics. The studies of pure natural products against colon cancer are now in focus. Another project is focused on bioengineering of circular proteins, so called cyclotides, to create new structure–activity relationships. Novel strategies are developed for efficient prediction and selection of organisms and molecules and bioinformatics tools to predict novel targets based on lateral gene transfer.

A scientific platform has been built in our long-term research on anti-inflammatory natural products as demonstrated in a number of publications and doctoral theses. Many different chemical structures have been discovered, and chemically and pharmacologically characterized using bioassay-guided isolation procedures. In vivo methods such as rat paw and mouse ear edema was used and later followed by in vitro enzyme and cell based methods. Two systems have been established to enable investigations of the effects of natural compounds on COX-2. The first method developed was an in vitro method suitable for measuring inhibition of COX-2 catalyzed prostaglandin E2 biosynthesis, based on scintillation proximity assay technology [14]. The second system comprises acell model, suitable for studying the effects of compounds on COX-2 and inducible nitric oxide synthase (iNOS) at different cellular levels, including the effects on mRNA, protein, prostaglandin E2, and nitric oxide levels [15].

In later years the project has developed towards enzyme inhibitors related to anti-tumor activity, especially in colon cancer. It has been shown that the process of inflammation and expression of cyclooxygenase-2 is important in colon carcinogenesis. Another important factor is diet. Many food python-chemicals have been shown to exert anti-inflammatory activity in vitro, and may act as cancer chemo-preventive agents [16, 17]. A vegetarian diet rich in python-chemicals may prevent colon carcinogenesis by affecting biochemical processes in the colonic mucosa. It has been shown that intact fecal water (water phase) samples from human volunteers significantly decreased prostaglandin production and COX-2 expression in colonic cells. NMR spectroscopy and multivariate data analysis were later used for further analysis of the composition of the fecal waters and to trace the COX-2 inhibiting activity [18, 19]. The wealth of different natural products with experimentally demonstrated COX inhibitory effects and an urge to understand and characterize their structural diversity was the starting point for the application of chirography in our natural products research.

The identification from chemo graphic analyses of some specific groups of compounds, including a set of cardiac glycosides, as being of prime importance was further established in a screening of a large number of natural products for activity against colorectal cancer where several cardiac glycosides showed significant activity. This activity was further confirmed in primary cells from colon cancer patients. Cardiac glycosides have been reported to exhibit cytotoxic activity against several different cancer types, but studies against colorectal cancer are lacking. Drugs for clinical treatment of colon cancer are usually used in combination to overcome the problem with drug resistance and to increase the activity. Therefore, selected cardiac glycosides were tested in combination with four
clinically relevant standard cyto-toxic drugs (5-fluorouracil, oxaliplatin, cisplatin, irinotecan) to screen for synergistic effects.

The combination of digoxin and oxaliplatin exhibited synergism including the otherwise highly drug resistant HT29 cell line [20]. In depth studies are now in progress necessary to understand these effects on a molecular level.

A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways, which provide evidence that the plant flavonoid fisetin can induce apoptosis and suppress the growth of colon cancer cells by inhibition of COX2- and Wnt/EGFR/NF-kappaB-signaling pathways and suggest that fisetin could be a useful agent for prevention and treatment of colon cancer [21]. The contribution of plukenetione A to the antitumor activity of Cuban propolis was assumed that plukenetione A contributes to the antitumor effect of Cuban propolis mainly by targeting to poiosomerase I as well as DNA polymerase[22]. Aberrant Wnt/beta-catenin signaling has recently been implicated in tumor genesis. On the basis of screening program targeting inhibition of TCF/beta-catenin transcriptional activity, a plant extract of Eleutherine palmfolia was selected as a hit sample. Activity-guided fractionations led to the isolation of 15 naphthalene derivatives (1-15), including 4 new glycosides, eleutherinosides B-E (1-4), and 10 of the 15 compounds showed strong activities with high viability among 293T cells, whose data showed that 2 and 9 inhibited the transcription of TCF/beta-catenin in SW480 colon cancer cells in a dose-dependent manner and selective cytotoxicity against three colorectal cancer cell lines. In addition, treatment with 9 led to a significant decrease in the level of nuclear beta-catenin protein, suggesting this reduction to have resulted in the inhibitory effect of 9 on the transcription of TCF/beta-catenin [23].

Penta-cyclic triterpene acids are known mainly for their anti-antigenic effects as well as their differentiation inducing effects. In particular, lupane-type triterpenes, such as botulin, botulnic acid and lupeol, display anti-inflammatory activities which often accompany immune modulation. Tri-terpene acids as well as triterpene mono-alcohols and diols also show an anti-oxidative potential. The pharmacological potential of triterpenes of the lupane, oleaneurane or urbane type for cancer treatment seems high; although up to now no clinical trial has been published using these tri-terpenes in cancer therapy. They provide a multi-target potential for coping with new cancer strategies. Whether this is an effective approach for cancer treatment has to be proven. Because various triterpenes are an increasingly promising group of plant metabolites, the utilization of different plants as their sources is of interest. Parts of plants, for example birch bark, rosemary leaves, apple peel and mistletoe shoots are rich in triterpenes and provide different triterpene compositions [24].

Advanced cancer is a multi-factorial disease that demands treatments targeting multiple cellular pathways. Chinese herbal cocktail which contains various phytochemicals may target multiple dys-regulated pathways in cancer cells and thus may provide an alternative/complementary way to treat cancers. Previously reported that the Chinese herbal cocktail Tien-Hsien Liguid (THL) can specifically induce apoptosis in various cancer cells and have immuno-modulating activity. Further, evaluated the anti-metastatic, anti-angiogenic and anti-tumor activities of THL [25]. Dietary grape seed extract (GSE) effectiveness in preventing
azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation. GSE-feeding inhibited AOM-induced cell proliferation but enhanced apoptosis in colon including ACF, together with a strong decrease in cyclin D1, COX-2, iNOS, and survivin levels and showed that GSE-feeding also decreased AOM-caused increase in beta-catenin and NF-kappaB levels in colon tissues[26]. Grifolin, a secondary metabolite isolated from the fresh fruiting bodies of the mushroom Albatrellus confluens, has been shown to inhibit the growth of some cancer cell lines in vitro by induction of apoptosis An apoptosis-related gene expression profiling analysis provided a clue that death-associated protein kinase 1 (dapk1) gene was up-regulated at least twofold in response to grifolin treatment in nasopharyngeal carcinoma cell CNE1. Further, investigated the role of DAPK1 in apoptotic effect induced by grifolin and observed that protein as well as mRNA level of DAPK1 was induced by grifolin in a dose-dependent manner in nasopharyngeal carcinoma cell CNE1. It was found that grifolin increased both Ser392 and Ser20 phosphorylation levels of transcription factor p53 protein, which could promote its transcriptional activity. Moreover, induced by grifolin, the recruitment of p53 to dapk1 gene promoter was confirmed to enhance markedly using EMSA and ChIP assays analysis. The involvement of DAPK1 in grifolin-induced apoptosis was supported by the studies that introducing siRNA targeting DAPK1 to CNE1 cells remarkably interfered grifolin-caused apoptotic effect as well as the activation of caspase-3. Grifolin induced up-regulation of DAPK1 via p53 was also observed in tumor cells derived from human breast cancer and human colon cancer. Up-regulation of DAPK1 via p53-DAPK1 pathway is an important mechanism of grifolin contributing to its ability to induce apoptotic effect. Since growing evidence found a significant loss of DAPK1 expression in a large variety of tumor types, grifolin may represent a promising candidate in the intervention of cancer via targeting DAPK1[27]. Five derivatives of the natural product sansalvamide A that are potent against multiple drug-resistant colon cancer cell lines were identified. These analogs share no structural homology to current colon cancer drugs, are cytotoxic at levels on par with existing drugs treating other cancers, and demonstrate selectivity for drug-resistant colon cancer cell lines over noncancerous cell lines. Thus, we have established sansalvamide A as a privileged structure for treating multiple drug-resistant colon cancers [28]. Anti-cancer activities of the ethanol extract of Ka-mi-kae-kyuk-tang (KMKKT) targeting angiogenesis, apoptosis and metastasis without any adverse effect on the body weight. This formula merits serious consideration for further evaluation for the chemoprevention and treatment of cancers of multiple organ sites[29]. The effect of aged garlic extract (AGE) on the growth of colorectal cancer cells and their angiogenesis, which are important microenvironmental factors in carcinogenesis. AGE (aged garlic extract) could prevent tumor formation by inhibiting angiogenesis through the suppression of endothelial cell motility, proliferation, and tube formation. AGE would be a good chemo-preventive agent for colorectal cancer because of its anti-proliferative activity on colorectal carcinoma cells and inhibitory activity on angiogenesis. Aged garlic extract (AGE) has manifold biological activities including immune-modulated and anti-oxidative effects. It is used as a major component of nonprescription tonics and cold-prevention medicines or dietary supplements [30, 31].

Transcription factor NF-kappaB is constitutively active in many human chronic inflammatory diseases and cancers. Epoxy quinone A monomer (EqM), a synthetic derivative of the nat-
ural product epoxyquinol A, has previously been shown to be a potent inhibitor of tumor necrosis factor-alpha (TNF-alpha)-induced activation of NF-kappaB [32]. EqM also effectively inhibits the growth of human leukemia, kidney, and colon cancer cell lines in the NCI’s tumor cell panel. Among six colon cancer cell lines, those with low amounts of constitutive NF-kappaB DNA-binding activity are generally more sensitive to growth inhibition by EqM. Therefore, EqM inhibits growth and induces cell death in tumor cells through a mechanism that involves inhibition of NF-kappaB activity at multiple steps in the signaling pathway.

Colorectal cancer, the second most frequent diagnosed cancer in the US, causes significant morbidity and mortality in humans. Over the past several years, the molecular and biochemical pathways that influence the development of colon cancer have been extensively characterized. Since the development of colon cancer involves multi-step events, the available drug therapies for colorectal cancer are largely ineffective. The radiotherapy, photodynamic therapy, and chemotherapy are associated with severe side effects and offer no firm expectation for a cure. Thus, there is a constant need for the investigation of other potentially useful options. One of the widely sought approaches is cancer chemoprevention that uses natural agents to reverse or inhibit the malignant transformation of colon cancer cells and to prevent invasion and metastasis [33]. Curcumin (diferuloylmethane), a natural plant product, possesses such chemopreventive activity that targets multiple signaling pathways in the prevention of colon cancer development [34]. Colon-targeting delivery of rhubarb extract, as a purgative, may prevent absorption of free anthraquinones in the upper gastrointestinal tract, thus improving clinical effects and lowering dosage [35, 36]. Chemopreventive effects of arctin, a lignin isolated from Arctium lappa (burdock) seeds, on the initiation or post initiation period of 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) induced mammary carcinogenesis in female rats and on 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)-associated hepatocarcinogenesis [37].

3. Plant peptides and proteins

Plant peptides and proteins may be considered an overlooked source for new chemical entities and novel bioactivities compared to low molecular natural products. The reason for this seems to be based on tradition and biased by the most commonly used techniques for natural products. However, during the last decades, the number of reported plant peptides has grown substantially, and the field is about to mushroom. In the form of Professor Gunnar Samuelsson’s’ pioneering studies of mistletoe toxins was reported [38]. In the mid 1990san attempt to assess plant peptides more broadly, with the design of an isolation protocol directly designed for their isolation [39]. One of the results of this effort was the discovery of a set of macrocyclic peptides in the plant family Violaceae [40]. Strikingly, the peptides we characterized in Violaceae were found to be nearly identical with one of the most intriguing examples of pharmacognostic research in general, and plant peptides in particular, namely the peptide Kalata B1. The discovery of Kalata B1 was based on the ethno-pharmacological use of the plant Oldenlandia affinis. It was experienced a high frequency of complicated deliveries due to the use of this plant, which was locally known as “Kalata-Kalata” [41].
tive women secretly used a decoction of this plant to facilitate childbirth, which they sipped as a tea but also applied directly at the birth canal [42]. It induced extremely strong uterine contractions, which sometimes developed into cervical spasms necessitating acute caesarean section.

The complete sequence and the cyclic structure was however not determined for more than 20 years later [43]. At the time of our report of the first cocktail of “palate-like” peptides in Violaceae, four similar peptides had been reported in the literature as the serendipitous discoveries of three independent groups. When including those peptides in a sequence comparison, i.e. the anti-HIV circulins A and B, the neurotensin binding inhibitor cyclo-psychotride A and the partially characterized violapeptide I, it was clear that they fell in two subgroups based on sequence similarity. Today, around 150 cyclotides have been reported from species of three plant families, Violaceae, Rubiaceae and Cucurbitaceae. The family Violaceae seems to be particularly rich in these proteins [44-47] and a single Violaceae species may contain more than 60 different cyclotides. It has been suggested that there might be 9,000 cyclotides in the Violaceae alone [48].

In addition to the amide bond that cyclizes the backbone, cyclotides contain three stabilizing disulfide bonds in a knotted arrangement, i.e. two disulfides form a ring together with their connecting protein backbone, which is threaded by the third disulfide [49-51].

CCK motif, and make cyclotides extraordinary stable protein structures [52]. Besides being uterotonic, anti-HIV, hemolytic and neurotensin binding inhibitory, the list now includes antimicrobial [53], antifouling [54], antihelmintic, molluscicidal, cytotoxic [55, 56]) and insecticidal activities. The latter effects are some of the most well studied and interesting effects: the cytotoxic effect together with the haemolytic effect have been the focus for detailed structure activity studies [57, 58], and the discovery of their insecticidal effect likely revealed cyclotides’ role in planta. Cyclotides’ mechanism of action is however yet unknown, but evidence is accumulating showing that membrane interactions followed by membrane pore or fissure formation are involved [59-61], which could provide an explanation to several of the reported effects.

Combined with the extraordinary CCK motif—with its conserved scaffold that can be dressed with variable loop sequences—the demonstrated biological activity of the cyclotides make them a first class target for protein engineering. To this end, inherent activities of native cyclotides can be reinforced or abolished, or new biologically active peptide epitopes can be grafted into the scaffold. For example, reinforcing the cytotoxic effect could potentially provide us with leads for anticancer drugs, or to completely remove that effect could provide us with an inert scaffold ideal for grafting. The first successful grafting of a biologically active epitope was reported just recently, showing proof of concept [62].

The success of these strategies relies on the ability of efficient methods for production of cyclotides and cyclotide mutants. Being gene products, cell based production systems seem promising, but although cyclotide producing plant cell cultures have been established [63], solid phase peptide synthesis is still the method of choice [64, 65]. Our knowledge about their biosynthesis is yet scarce. We know the structural arrangement of the cyclotide precur-
isor from cDNA, and that an asparaginyl endo-peptidase has a likely role for cleaving at the N terminal side of the mature peptide and that protein disulfide isomerasers seem to play a role for their successful folding. However, the order of the events to produce mature cyclotides is not yet known, i.e. if disulfide bonds are formed before or after excision and ligation, and nothing is known about how these processes are controlled. In the perspective of exploiting the cyclotide scaffold for engineering of bioactive peptides, the possibility of farming designed molecules in plantar promises to be the optimal solution; the way there is still long though.

Matrix metalloproteinase (MMP) are zinc-dependent endopeptidases that mediate numerous physiologic and pathologic processes, including matrix degradation, tissue remodeling, inflammation, and tumor metastasis. To develop a vaccine targeting stromal antigens expressed by cancer-associated fibroblasts, it was focused on MMP11 (or stromelysin 3). MMP11 expression correlates with aggressive profile and invasiveness of different types of carcinoma [66]. Overexpression of IL-12 and IL-23, which share the p40 subunit, has been implicated in the pathogenesis of Crohn’s disease. Targeting these cytokines with monoclonal antibodies has emerged as a new and effective therapy, but one with adverse reactions [67]. Intestinal fibrosis and stricture formation are major complications of inflammatory bowel disease (IBD), for which there are currently few effective treatments. It was investigated whether targeting transforming growth factor-beta1 (TGF-beta1), a key profibrotic mediator, with a peptide-based virus-like particle vaccine would be effective in suppressing intestinal fibrosis by using a mouse model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced chronic colitis [68]. Neutralization of macrophage migration inhibitory factor (MIF) by anti-MIF antibody reduces intestinal inflammation in mice. Anti-MIF autoantibody induced by DNA vaccine targeting MIF protects mice against experimental colitis [69].

Overexpression of the proto-oncogene c-Myb occurs in more than 80% of colorectal cancer (CRC) and is associated with aggressive disease and poor prognosis. To test c-Myb as a therapeutic target in CRC, which devised a DNA fusion vaccine to generate an anti-CRC immune response. c-Myb, like many tumor antigens, is weakly immunogenic as it is a "self" antigen and subject to tolerance [70]. Four novel oral DNA vaccines provide protection against melanoma, colon, breast, and lung carcinoma in mouse models. Vaccines are delivered by attenuated Salmonella typhimurium to secondary lymphoid organs and respectively target vascular endothelial growth factor receptor-2, transcription factor Fos-related antigen-1, anti-apoptosis protein survivin, and Legumain, an asparaginyl endopeptidase specifically overexpressed on tumor-associated macrophages (TAMs) in the tumor microenvironment (TME). These vaccines are all capable of inducing potent cell-mediated protective immunity against self-antigens, resulting in marked suppression of tumor growth and dissemination. Key mechanisms induced by these DNA vaccines include efficient suppression of angiogenesis in the tumor vasculature and marked activation of cytotoxic T cells, natural killer cells, and antigen-presenting dendritic cells [71]. Shigellosis is a major form of bacillary dysentery caused by Shigella infection. Shigella ribosome-based vaccines (SRV), considered among the potent vaccine candidates, are composed of O-antigen and ribosome isolated from S. flexneri 2a. The immunogenicity and protective efficacy of SRV was investi-
gated and mice were vaccinated with SRV via the intranasal route. Interestingly, robust levels of Shigella-derived LPS-specific IgG and IgA Abs and antibody-forming cells were elicited in systemic and mucosal compartments following two intranasal administrations of SRV. Groups of mice receiving intranasal SRV developed milder pulmonary pneumonia upon challenge with virulent S. flexneri 2a than did those receiving parenteral SRV [72].

Over the past several years it has become apparent that the tumor stroma represents a significant target for anti-cancer therapies. Therefore we evaluated the strategy of targeting the tumor stroma with a novel DNA vaccine encoding murine platelet derived growth factor receptor-beta (mPDGFRbeta). Immunization with this vaccine induced cytotoxic lysis of mPDGFRbeta-expressing target cells and protected mice from the growth and dissemination of murine colon, breast and lung carcinoma. Furthermore, this novel vaccine suppresses angiogenesis in vivo and reduces the numbers of tumor-associated, mPDGFRbeta-expressing pericytes as suggested by a decrease in intra-tumor expression of mPDGFRbeta and NG2 [73]. Viral vectors are under development for anticancer therapy. As they can infect tumors and activate the immune system, viral vectors may directly destroy cancers (oncolysis), deliver genes with antitumor activity directly to the cancer cells, or act as cancer vaccines. Better insights into the biology of the various vectors in use (e.g., poxvectors, adenovirus, adeno-associated virus, retrovirus, Newcastle disease virus) are making it possible to engineer viruses that are more tumor-specific, efficient at tumor infection, and which have enhanced safety due to incorporation of safeguards should dissemination occur [74].

Colorectal carcinoma is a leading cause of cancer-related mortality. Despite the introduction of new cytotoxic drugs, improved surgical and radiotherapeutic techniques, a large proportion of colorectal carcinomas remain incurable. New targeted therapeutic strategies, including immunotherapy, are being explored as complementary treatments. Recent advances in immunology and molecular biology have opened new avenues for the clinical testing of rationally designed vaccination strategies against cancer [75]. The use of retrogen plasmid-based vaccine technology to break tolerance and to generate a robust, dose-dependent antibody response against the self cancer antigen, survivin. This phenomenon is due to the incorporation of the survivin antigen into the retrogen system rather than to some peculiarity unique to survivin. In contrast to other genetic immunization methods designed to produce antibody responses, the retrogen system results in a broad range of antibody isotypes, indicative of both a Th-1 and a Th-2 CD4+ response. Additional evidence of a Th-1 response is demonstrated by tumor growth inhibition in a mouse model of colon cancer metastasis [76].

An efficient strategy based on a fully synthetic dendrimeric carbohydrate display (multiple antigenic glycolpeptide; MAG) to induce anti carbohydrate antibody responses for therapeutic vaccination against cancer was developed. The superior efficacy of the MAG strategy over the traditional keyhole limpet hemocyanin glycolconjugate to elicit an anticarbohydrate IgG response against the tumor-associated Tn antigen was shown. The influence of the glycolic carrier elements of such a tumor antigen for their recognition by the immune system was influenced. Finally, we additionally developed the MAG system by introducing promiscuous HLA-restricted T-helper epitopes and performed its immunological evaluation
in nonhuman primates. MAG:Tn vaccines induced in all of the animals strong tumor-specific anti-Tn antibodies that can mediate antibody-dependent cell cytotoxicity against human tumor [77].

Overcoming immune tolerance of tumor angiogenesis should be useful for adjuvant therapy of cancer, which hypothesized that vaccination with autologous endothelium would induce an autoimmune response targeting tumor angiogenesis. The effect of autologous with a vaccine of glutaraldehyde-fixed murine hepatic sinusoidal endothelial cells (HSEs) was more pronounced than that of xenogeneic human umbilical vein endothelial cells (HUVECs), which were tested in the same experimental setting. Its results suggest that vaccination with autologous endothelium can overcome peripheral tolerance of self-angiogenic antigens and therefore should be useful for adjuvant immunotherapy of cancer [78].

Tumor cells are elusive targets for immunotherapy due to their heterogeneity and genetic instability. Here, a novel, oral DNA vaccine that targets stable, proliferating endothelial cells in the tumor vasculature rather than tumor cells was described. Targeting occurs through upregulated vascular-endothelial growth factor receptor 2 (FLK-1) of proliferating endothelial cells in the tumor vasculature. This vaccine effectively protected mice from lethal challenges with melanoma, colon carcinoma and lung carcinoma cells and reduced growth of established metastases in a therapeutic setting. CTL-mediated killing of endothelial cells indicated breaking of peripheral immune tolerance against this self antigen, resulting in markedly reduced dissemination of spontaneous and experimental pulmonary metastases. Angiogenesis in the tumor vasculature was suppressed without impairment of fertility, neuromuscular performance or hematopoiesis, albeit with a slight delay in wound healing [79].

The HER-2/neu oncogenic protein is a well-defined tumor antigen. HER-2/neu is a shared antigen among multiple tumor types. Patients with HER-2/neu protein-overexpressing breast, ovarian, non-small cell lung, colon, and prostate cancers have been shown to have a pre-existing immune response to HER-2/neu. No matter what the tumor type, endogenous immunity to HER-2/neu detected in cancer patients demonstrates two predominant characteristics. First, HER-2/neu-specific immune responses are found in only a minority of patients whose tumors overexpress HER-2/neu. Secondly, immunity, if detectable, is of low magnitude. These observations have led to the development of vaccine strategies designed to boost HER-2/neu immunity in a majority of patients. HER-2/neu is a non-mutated self-protein, therefore vaccines must be developed based on immunologic principles focused on circumventing tolerance, a primary mechanism of tumor immune escape [80].

Listeria monocytogenes is an intracellular organism that has the unusual ability to live in the cytoplasm of the cell. It is thus a good vector for targeting protein antigens to the cellular arm of the immune response. Here, a model system, consisting of colon and renal carcinomas that express the influenza virus nucleoprotein and a recombinant L. monocytogenes that secretes This antigen, to test the potential of this organism as a cancer immunotherapeutic agent, which show that this recombinant organism can not only protect mice against lethal challenge with tumour cells that express the antigen, but can also cause regression of established macroscopic tumours in an antigen-specific T-cell-dependent manner.
An efficient strategy for the targeting of anti-tumor effector cells were prepared bispecific antibody (BsAb) containing anti-CD3 and an anti-c-erbB-2 proto-oncogene product. A trophoblast cell surface antigen has been characterized by a monoclonal antibody (mAb) 5T4, raised following immunization with solubilized wheat germ agglutinin binding glycoproteins from human syncytiotrophoblast plasma membrane (StMPM) [81].

4. Plant peptides and proteins targets and regulation of this important pathway

Aberrant activation of the canonical Wnt/beta-catenin pathway occurs in almost all colorectal cancers and contributes to their growth, invasion and survival. Phospholipase D (PLD) has been implicated in progression of colorectal carcinoma. However, an understanding of the targets and regulation of this important pathway remains incomplete and besides, relationship between Wnt signaling and PLD is not known [82]. Fibroblast activation protein is a product overexpressed by tumor-associated fibroblasts (TAF) and is the predominant component of the stoma in most types of cancer. Tumor-associated fibroblasts differ from normal adult tissue fibroblasts, and instead resemble transient fetal and wound healing-associated fibroblasts. Tumor-associated fibroblasts are critical regulators of tumor genesis, but differ from tumor cells by being more genetically stable [83]. Capability of human adipose tissue-derived mesenchyme stem cells (AT-MSC) to serve as cellular vehicles for gene-directed enzyme prodrug molecular chemotherapy. Yeast fusion cytosine deaminase: uracil phosphoribosyltransferase expressing AT-MSC (CD y-AT-MSC) combined with systemic 5-fluorocytosine (5FC) significantly inhibited growth of human colon cancer xenografts [84].

Dendritic cells (DCs), pulsed with the respective endothelium lysates significantly inhibited the growth of subcutaneous tumors as well as pulmonary metastases in mice, and their anti-tumor effect was superior to that of unparsed DCs. Immunohistopathological analysis showed significant decrease in the mean vascular density of tumors, correlating well with the extent of tumor inhibition. In vitro analysis of splenocytes isolated from immunized mice revealed an induction of cytotoxic T lymphocytes and activation of natural killer cells, with a lytic activity against activated endothelium but not tumor cells. In addition, antibodies reacting with activated endothelium [85]. Crohn’s disease (CD) is an inflammatory bowel disease that is associated with several changes in the immune system, including an increased number of infiltrating macrophages. These macrophages release a variety of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) which are critically involved in the onset and the development of CD. The present study was performed to explore the initial involvement of macrophages in the development of T-cell-mediated chronic colitis [86].

Tumor-associated fibroblasts are key regulators of tumor genesis. In contrast to tumor cells, which are genetically unstable and mutate frequently, the presence of genetically more stable fibroblasts in the tumor-stromal compartment makes them an optimal target for cancer immunotherapy. These cells are also the primary source of collagen type I, which contrib-
utes to decreased chemotherapeutic drug uptake in tumors and plays a significant role in regulating tumor sensitivity to a variety of chemotherapies [87].

Conventional anticancer therapy using cytotoxic drugs lacks selectivity and is prone to toxicity and drug resistance. Anticancer therapies targeting aberrant growth factor receptor signaling are gaining interest. The erbB receptor family belongs to the type I, the receptor tyrosine kinases class, and comprises EGFR, HER-2, HER-3, and HER-4. It has been targeted for solid tumor therapy, including breast, ovarian, colon, head-and-neck, and non-small-cell lung cancers. Structural aspects of this class of growth factor receptors, their oncogenic expression, and various pharmacological interventions including biological products and small molecules that inhibit these enzymes [88].

Recent reports of tumor regression following delivery of autologous tumor antigen-pulsed DCs suggest that defective antigen presentation may play a key role in tumor escape. Here, it is shown in two different murine tumor models, CT26 (colon adenocarcinoma) and B16 (melanoma), that the number and activation state of intratumor DCs are critical factors in the host response to tumors [89]. Epidermal growth factor receptor (EGFR), a member of a family of membrane receptors with tyrosine kinase activity, is emerging as a target candidate for anti-cancer therapy, due to its overexpression in many carcinomas and its relationship with several hallmark properties of malignant behavior such as continuous cell proliferation, escape from apoptosis, cell migration and angiogenesis. Specially appealing is the overexpression of EGFR in tumors such as lung, colon, kidney and head and neck carcinomas which are mostly resistant to current chemotherapy [90].

It was identified an organic solute transporter (OST) that is generated when two novel gene products are co-expressed, namely human OSTalpha and OSTbeta or mouse OSTalpha and OSTbeta. The results also demonstrate that the mammalian proteins are functionally complemented by evolutionarily divergent OST alpha-OST beta proteins recently identified in the little skate, Raja erinaceus, even though the latter exhibit only 25-41% predicted amino acid identity with the mammalian proteins. Human, mouse, and skate OSTalpha proteins are predicted to contain seven trans membrane helices, whereas the OSTbeta sequences are predicted to have a single trans membrane helix. co-expression is not required for proper membrane targeting. Interestingly, OSTalpha and OSTbeta mRNAs were highly expressed and widely distributed in human tissues, with the highest levels occurring in the testis, colon, liver, small intestine, kidney, ovary, and adrenal gland [91]. Human mucin 1 (MUC1) is an epithelial mucin glycoprotein that is overexpressed in 90% of all adenocarcinomas including breast, lung, pancreas, prostate, stomach, colon, and ovary. MUC1 is a target for immune intervention, because, in patients with solid adenocarcinomas, low-level cellular and humoral immune responses to MUC1 have been observed, which are not sufficiently strong to eradicate the growing tumor [92]. Efficient T cell priming by GM-CSF and CD40 ligand double-transduced C26 murine colon carcinoma is not sufficient to cure metastases in a therapeutic setting [93].

A new therapy against colon cancer was developed and investigated two kinds of strategy using a cancer-specific approach. First, employed the Cre/loxP regulation system to enhance the specific expression by carcinoembryonic antigen (CEA) promoter in CEA-producing tu-
mor cells, and examined whether sufficient enhancement to transcriptional activity of CEA promoter, which maintains its specificity in vitro and in vivo, could be obtained. Next, using dendritic cells pulsed with HLA-A24 epitope peptides of CEA, we performed a Phase I study of active immunotherapy in patients with advanced colon cancer. These results suggest that the newly developed therapy for colon cancer is a promising strategy; however, minor modification may be necessary [94]. Serum gastrin is known to be elevated in patients with liver-metastasizing colon cancer; thus, cholecystokinin (CCK) B/gastrin receptors may also be up-regulated. A liver-invasive model of colon cancer was established with the human colonic cell line C170HM2, which expresses the CCKB/gastrin receptor at both the gene and protein level. An antiserum has been derived that is directed against the NH2-terminal 17 amino acids of the human CCKB/gastrin receptor coupled to diphtheria toxoid. The peptide was denoted gastrin receptor protein (GRP) 1[95]. 2B1 is a bispecific murine monoclonal antibody (BsMAb) with specificity for the c-erbB-2 and Fc gamma RIII extracellular domains. This BsMAb promotes the targeted lysis of malignant cells overexpressing the c-erbB-2 gene product of the HER2/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the Fc gamma RIII A isoform [96-98].

5. Methods of targeting colon drug delivery by natural products

The various targeting methods to the colon include coating with pH dependent polymers, degradation by bacteria, specially azo-cross linked polymers and certain polysaccharides such as pectin and guar gum are good carriers for formulation, which are presented in Table 1. To achieve successful colonic delivery, a drug needs to be protected from absorption and the environment of the upper gastrointestinal tract and then be released into the proximal colon, which is considered the optimum site for colon targeted delivery of natural compounds. Colon targeting is naturally of value for the treatment of diseases of colon such as Chron’s disease, ulcerative colitis, and colorectal cancer.

6. Classification of targeting colon drug delivery system

Colon targeting drug delivery system is classified into three categories greatly and the relevant developed systems are published in many reports.

Firstly, Local Therapy - Higher local drug level can be achieved while minimizing side effects that occur due to the release in the upper GIT. The system can treat local disease effectively such as constipation, irritable bowel disease and colon cancer.

Secondly, Delayed Onset - Maximal plasmalevel can be achieved in the morning hours after bedtime administration by delayed onset of the system to adjust the circadian variations in the signs and symptoms of disease such asrheumatoid arthritis, hypertension and etc.
Thirdly, Protein and Peptide Delivery - The colon is a "friendlier" environment for proteins, peptides and vaccines compare to the upper GIT. Clinically relevant bioavailability may be achieved if the drugs can be protected from the upper GIT.

7. Local targeting colon drug delivery

It was evaluated that colon targeting characteristic of Kuikang colon targeted pellets (KCP) with determination of residual baicalin and baicalein concentration in gastrointestinal tract (GIT). The major challenges in targeting drug to various parts of the gastrointestinal tract include control of drug release with respect to its environment and transit time. These two variables should be taken into consideration in designing a rational colonic drug delivery system. To this end, a swelling matrix core containing pectin, hydroxylpropyl methylcellulose (HPMC), microcrystalline cellulose and 5-aminosalicylic acid was developed. This was subjected to a dual coating operation: an inner pH-sensitive enteric and an outer semi-permeable membrane coat with a pore former [99-101].

A pectin-hydroxylpropyl methylcellulose coating was compressed onto core tablets labeled with 4MBq (99m)Tc-DTPA. Prolonged residence at the ICJ is assumed to have increased hydration of the hydrogel layer surrounding the core tablet. Forces applied as the tablets progressed through the ICJ may have disrupted the hydrogel layer sufficiently to initiate radiolabel release. Inadequate prior hydration of the hydrogel layer preventing access of pectinolytic enzymes and reduced fluid availability in the TC may have retarded tablet disintegration and radiolabel diffusion.

8. Delayed onset for targeting colon drug delivery

A multiparticulate system having pH-sensitive property and specific enzyme biodegradability for colon-targeted delivery of metronidazole was developed [102]. Pectin microspheres were prepared using emulsion-dehydration technique. These microspheres were coated with Eudragit(R) S-100 using oil-in-oil solvent evaporation method. The in vivo studies were also performed by assessing the drug concentration in various parts of the GIT at different time intervals which exhibited the potentiality of formulation for colon targeting. Hence, it can be concluded that Eudragit coated pectin microspheres can be used for the colon specific delivery of drug.

Designing pH-sensitive, polymeric nanoparticles of curcumin, a natural anti-cancer agent, for the treatment of colon cancer, which enhance the bioavailability of curcumin, simultaneously reducing the required dose through selective targeting to colon [103]. Eudragit S100 was chosen to aid targeting since the polymer dissolves at colonic pH to result in selective colonic release of the entrapped drug. Solvent emulsion-evaporation technique was employed to formulate the nanoparticles. The combined influence of 3 independent variables in the compression coated tablet of mesalamine [104] for ulcerative colitis. A 3-factor, 3-level
Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were: percentage of polymers (pectin and compritol ATO 888) in compression coating (X(1)), coating mass (X(2)) and coating force (X(3)). Fifteen batches were prepared and evaluated for percent of drug released in 5 h (Y(5)), time required for 50 % mesalamine to dissolve (t(50)) with rat cecal (RC) content and without rat cecal content (t(50)), percent of drug released in 24 h in the presence of rat cecal content (Y(24) with RC) [105].

The colon specificity of novel natural polymer kaya gum and compare with guar gum. Release profile of tablets was carried out in presence and absence of rat cecal contents. The fast disintegrating core tablets of budesonide, were initially prepared by direct compression technique. Later, these tablets were coated with kaya gum or guar gum. After suitable pre compression and post compression evaluation, these tablets were further coated using Eudragit L-100 by dip coating technique [106]. Enteric-coated calcium pectinase microspheres (MS) aimed for colon drug delivery have been developed, by using theophylline as a model drug. The influence of pectin type (animated or non-animated) and MS preparation conditions (CaCl₂ concentration and cross-linking time) was investigated upon the drug entrapment efficiency and its release behavior. Pectin/ethyl cellulose-film-coated pellets of 5-fluorouracil (5-FU) [107] for colonic targeting were characterized. The pellet cores were coated to different film thicknesses with three different pectinethyl cellulose formulations using a fluidized bed coater [108]. The gastrointestinal (GI) transit of coated pellets was determined by counting the percentage of coated pellets in the GI lumen by celiotomy at certain times after oral administration. 5FU was administered to rats at a dose of 15 mg kg⁻¹. The toxicity of 5-FU in the GI tract was evaluated using histological examination. The 1:2 ratio pectin:ethyl cellulose-coated pellets with 30% total weight gain (TWG-30%) produced more satisfactory drug-release profiles in the simulated gastric, intestinal and colonic fluids [109]. Most of the coated pellets were eliminated from the stomach in 2 h, moved into the small intestine after 2-4 h, and reached the large intestine after 4 h.A novel colon targeted tablet formulation was developed using natural polysaccharides such as chitosan and guar gum as carriers and diltiazem hydrochloride as model drug [110]. The prepared blend of polymer-drug tablets were coated with two layers, inulin as an inner coat followed by shellac as outer coat and was evaluated for properties such as average weight, hardness and coat thickness. In vitro release studies of prepared tablets were carried out for 2 h in pH 1.2 HCl buffers, 3 h in pH 7.4 phosphate buffer and 6 h in simulated colonic fluid (SCF) in order to mimic the conditions from mouth to colon. Coated micro-pellets of pH-dependent and enzyme-dependent kangfuxin colon targeting delivery system were prepared; to make them go to colon, then release, educe partial effect [111].

The ingredients for preparing the micro-pellets are 125% starch +2% CMC-Na, and add 30% ethanol to be binder, pellets were coated with Eudragit S100 to prepare pH-dependent and pectin-HPMC to prepare enzyme-dependent colon targeting micro-pellets.

Guar gum/ethyl cellulose mix coated pellets for potential colon-specific drug delivery were designed [112, 113]. The coated pellets, containing 5-fluorouracil as a model drug, were prepared in a fluidized bed coater by spraying the aqueous/ethanol dispersion mixture of guar
gum and ethyl cellulose. The lag time of drug release and release rate were adjustable by changing the ratio of guar gum to ethyl cellulose and coat weight gain. In order to find the optimal coating formulation that was able to achieve drug targeting to the colon and concluded that mixed coating of guar gum and ethyl cellulose is able to provide protection of the drug load in the upper gastrointestinal tract, while allowing enzymatic breakdown of the hybrid coat to release the drug load in the colon. A pectin-based colon specific delivery system bearing 5-fluorouracil (5-FU) was developed for effective delivery of drug to the colon. Calcium pectinate gel (CPG) beads were prepared by ion tropic gelation method followed by enteric coating with Eudragit S-100 [114, 115]. The CPG beads formed were spherical with smooth surfaces. Eudragit S-100 coated calcium pectinate beads delivered most of its drug load (93.2+/−3.67%) to the colon after 9 h, which reflects its targeting potential to the colon. It is concluded that orally-administered 5-FU loaded Eudragit S-100 coated calcium pectinate beads can be used effectively for the specific delivery of drug to the colon. Colon-targeted drug delivery systems for 5-fluorouracil using pectin combined with ethyl cellulose as a film coat with fluidized bed coater were developed. Pellets (0.8-1.0 mm in diameter) containing 40% 5-fluorouracil and 60% microcrystalline cellulose were prepared by extrusion and spherization. Eudragit-coating of pectin microspheres was performed by oil-in-oil solvent evaporation method using coat: core ratio (5:1). The release profile of FU from Eudragit coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of FU.

Guar gum-based matrix tablets of rofecoxib for their intended use in the chemoprevention of colorectal cancer were developed and evaluated [116]. Matrix tablets containing 40% (RXL-40), 50% (RXL-50), 60% (RXL-60) or 70% (RXL-70) of guar gum were prepared by wet granulation technique. Colon delivery of beta-lactamases by pectin beads aiming to degrade residual beta-lactam antibiotics, in order to prevent the emergence of resistant bacterial strains [115]. Pectin beads were prepared according to inotropic gelation method using CaCl₂ as a gelling agent [117]. Particles were then washed and soaked in polyethyleneimine (PEI). Coating beads with PEI considerably improved their stability in simulated intestinal medium.

9. Polysaccharides as a strategy for targeting colon drug delivery

A variety of delivery strategies and systems have been proposed for colonic targeting. These generally rely on the exploitation of one or more of the following gastrointestinal features for their functionality: pH, transit time, pressure or micro flora. Coated systems that utilise the pH differential in the gastrointestinal tract and prodrugs that rely on colonic bacteria for release have been commercialized. Both approaches have their own inherent limitations. Many systems in development have progressed no further than the bench, while others are expensive or complex to manufacture, or lack the desired site-specificity. The universal pol-
ysaccharide systems appear to be the most promising because of their practicality and exploitation of the most distinctive property of the colon, abundant micro flora.

Recent research into the utilization of the metabolic activity and the colonic microenvironment in the lower gastrointestinal tract has attained great value in the design of novel colon-targeted delivery systems based on natural biodegradable polymers. In the current articles, special emphasis has been placed on polysaccharide systems, with minimal chemical modification, that have been exploited for colon targeting. These polysaccharide based encapsulation and targeted delivery systems are envisaged to have an immense potential for the development of food/nutraceutical formulations for colon-based diseases, including colorectal cancer [118]. Pectin-ketoprofen (PT-KP) prodrug with the potential for colon targeted delivery has been evaluated and showed KP distributes mainly in stomach, proximal small intestine and distal small intestine. However, KP released from PT-KP mainly distributes in cecum and colon. Therefore, this approach suggests that PT-KP prodrug has a good colon targeting property [119].

Compression coatings for target drug delivery to the colon using indometacin (a water insoluble drug) and paracetamol (a water soluble drug) as model drugs were evaluated. The core tablets were compression-coated with 300 and 400 mg of 100% kayas gum, 100% albizia gum and a mixture of kaya and albizia gum (1:1). Colon targeted drug delivery systems were developed for tinidazole using guar gum as a carrier in the treatment of amoebiasis. Fast-disintegrating tinidazole core tablets were compression-coated with 55, 65 and 75% of guar gum [120]. Colon-targeted drug delivery systems for ornidazole [121] using guar gum as a carrier are developed. The core formulation containing ornidazole was directly compressed. Compression-coated tablets of ornidazole containing various proportions of guar gum in the coat were prepared. Compression-coated ornidazole tablets with either 65% (OLV-65) or 75% (OLV-75) of guar gum coat are most likely to provide targeting of ornidazole for local action in the colon owing to its minimal release of the drug in the first 5 hr. The ornidazole compression-coated tablets showed no change in physical appearance, drug content, or in dissolution pattern after storage at 40 degrees C/75% relative humidity for 6 months [122-125].

Novel tablet formulations for site-specific delivery of 5-fluorouracil to the colon without the drug being released in the stomach or small intestine using guar gum as a carrier were developed. Fast-disintegrating 5-fluorouracil core tablets were compression coated with 60% (FHV-60), 70% (FHV-70) and 80% (FHV-80) of guar gum [126]. Oral colon-targeting drug delivery systems for celecoxib using guar gum as a carrier were developed. Matrix tablets containing various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder. Colon targeted drug delivery systems for metronidazole using guar gum as a carrier were developed. Matrix, multilayer and compression coated tablets of metronidazole containing various proportions of guar gum were prepared [127]. The influence of metronidazole and tinidazole on the usefulness of guar gum, a colon-specific drug carrier based on the metabolic activity of colonic bacteria, using matrix tablets of albendazole containing 20% of guar gum as a model formulation is reported. Colon targeted drug delivery systems for mebendazole using guar gum as a carrier were developed. Matrix tablets con-
taining various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder [128, 129].

Controlling the delivery of drugs to different regions of the colon remains an elusive goal—was to define the diurnal variation in colonic transit and show how this influences the colonic distribution and residence time of different formulations given either in the morning or evening. Colonic transit of small particulates and a large capsule was measured during nocturnal sleep and daytime wakefulness. Sleep delays colonic transit and large capsules travel faster than dispersed small particles. However, substantial inter-individual variability in transit makes targeting specific regions of the human colon unreliable with either dispersed or single unit formulations [130].

Targeting of drugs to the colon, following oral administration, can be accomplished by the use of modified, biodegradable polysaccharides as vehicles. In a previous study, a cross-linked low swelling guar gum (GG) hydrogel was synthesized by reacting it with trisodium trimetaphosphate (STMP). In the present study the functioning of GG crosslinked products (GGP) as possible colon-specific drug carriers was analyzed by studying (a) the release kinetics of pre-loaded hydrocortisone from GGP hydrogels into buffer solutions with, or without GG degrading enzymes (alpha-galactosidase and beta-mannanase) and (b) direct measurements of the polymers' degradation [131]. Calcium pectinate preparations for drug delivery to the colon were investigated and highlight the value of scintigraphy in focusing the development strategy for colonic targeting preparations.

One of the review articles concluded that polysaccharide-based colon-targeted drug delivery systems are effective when they are precisely activated by the physiological conditions of the colon. Absence of enzymes during colonic disorders might hinder the activation of the delivery system. To guarantee delivery of the drug to the colon, it is preferable to combine polysaccharides with enteric or cellulose polymers [132]. The approach that is based on the formulation of natural polysaccharides has been used as tools to deliver the drugs specially to the colon as described in Table 1 and Table 2.

These polysaccharides remain intact in the physiological environment of stomach and small intestine but once the dosage form enters into colon, it is acted upon by polysaccharides, which degrades the polysaccharide and release the drug into the vicinity of bio-environment of colon (Table 3). As shown in Figure 1 the designed drug delivery systems have been developed that are based on the principle to prevent release of drug until 3-4 h after leaving the stomach, which are correspond to blood concentration profile as illustrated in Figure 2.

With the respect to natural products improved drug delivery systems are required for drugs currently in use to treat localized disease of the colon. The advantages of targeting drugs specially to the diseased colon are reduced incidence of systemic side effects, lower dose of drug, supply of the drug to the bio phase only when it is required and maintenance of the drug its intact form as close as possible to target site. Thus, the natural and modified properties of polysaccharides that are responsible for their colon targeting abilities. Among the different approaches used, polysaccharides that are precisely activated by the physiological
conditions of the colon hold great promise, as they provide improved site specificity and meet the desired therapeutic needs.

Figure 1. Dissolution profile from microbial degradable colon delivery system coated with polysaccharide.

Figure 2. Blood plasma concentration profile of nifedipinetablet(top) and soft(bottom) capsule verse disintegration time
### Table 1. Main Structural Features of Polysaccharides

<table>
<thead>
<tr>
<th>Polysaccharides</th>
<th>Main Chains</th>
<th>Side Chains</th>
<th>Bacterial Species</th>
</tr>
</thead>
</table>
| Pectin          | 1) α-1,4 D-garactouronic acid  
                2) 1,2 -L-rhamnose | D-galactose  
                L-arabinose | Bacteroides  
                Bifidobacteria  
                Eubacteria |
| Guar Gum        | 1) α-1,4 D-mannose  
                2) α-1,3 or α-1,4 D-galactose | α-1,6 D-galactose | Bacteroides  
                Ruminococci |
| Amylose         | α-1,4 D-glucose | - | Bacteroides |
| Chitosan        | Deacetylated β-1,4 N-acetyl-D-glucosamine | - | Bacteroides |
| Chondroitin Sulfate | 1) β-1,3 D-glucuronic acid  
                2) N-acetyl-D-glucosamine | - | Bacteroides |
| Cyclodextrine   | α-1,4 D-glucose | - | Bacteroides |
| Dextran         | α-1,6 D-glucose  
                α-1,3 D-glucose | - | Bacteroides |
| Xylan           | β-1,4 D-xylene  
                β-1,3 L-arabinose | - | Bacteroides  
                Bifidobacteria |

### Table 2. Microbial Degradable Colon Delivery System.

<table>
<thead>
<tr>
<th>System</th>
<th>CoLar ®</th>
<th>CSDS</th>
<th>Colon Delivery System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developer</td>
<td>Allyzyme (U.K)</td>
<td>Samyang Co., ( Korea )</td>
<td>Perio ( Israel )</td>
</tr>
</tbody>
</table>
| Composition | Amylose+Ethylcellulose Coating | Tablet or Soft capsule coated with Polysaccharide | 1) Fast release: Ca.P + P  
                2) Slow release:Ca.P + GG |
| Dosage form | Coated Tablet | Coated Tablet  
                Coated Soft Capsule | Matrix Tablet |
| Drug | Rensapride | Budesonide, Mesalazine  
                Diclofenac | Prednisolone  
                Theophylline  
                Diclofenac  
                Insulin |
| Indication | Constipation  
                Predominant  
                I.B.S. | I.B.D.  
                Rheumatoid Arthritis | Rheumatoid Arthritis  
                Diabetes |
| Stage | Phasell (Japan)  
                Phasell (U.K) | System Development | System Development |
<p>| Licensee | S.K.B | Now discontinued for internal reason | Alpharma Co., |</p>
<table>
<thead>
<tr>
<th>System</th>
<th>Targit ®</th>
<th>Chronotopic ®</th>
<th>CTDC ®</th>
<th>Colon Delivery System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developer</td>
<td>DanBio System (U.K.)</td>
<td>Poli Chemical Industry (Italy)</td>
<td>Tanabe Seiyaku (Japan)</td>
<td>Chiesi (Italy)</td>
</tr>
</tbody>
</table>
| Composition  | Starch Capsule + Enteric Coating | 1) 1st: Core Tablet  
2) 2nd: Low-Viscosity HPMC  
3) 3rd: Eudragit | 1) 1st layer: Eudragit L  
2) 2nd layer: HPMC  
3) 3rd layer: Eudragit E | Enteric (Eudragit–S) Coating |
| Dosage form  | Coated Capsule    | Coated tablet      | Coated Capsule     | Coated Tablet          |
| Drug         | Mesalazine        | Mesalazine         | Prednisolone       | Beclomethasone Dipropionate |
| Indication   | I.B.D.            | I.B.D.             | -                  | I.B.D.                 |
| Stage        | System Development | Phase II (Italy)   | System Development | Phase III (Italy)      |
| Licensee     | Western Pharma.   | -                  | -                  | -                      |

Table 3. Time-Controlled &pH-Controlled Colon Delivery System

10. Protein and peptide drug targeting colon delivery

Targeted delivery to the gastrointestinal tract requires a multi-disciplinary approach to research involving contributions from polymer and material scientists, gastroenterologists, pharmaceutical scientists and technologists. Intestinal delivery is important not only for drugs that act locally, but also for those with systemic activity [133, 134]. In particular, there is considerable interest in the oral delivery of peptides and it is felt that the colon may provide an advantageous absorption site for such molecules. The different targeting mechanisms available to the pharmaceutical scientist to provide site-specific delivery in the gastrointestinal tract will be critically assessed. Delivery systems and targeting agents, which are being developed for the delivery of drugs, may also be exploited for the delivery of vaccines, since many of the delivery problems are common to both areas. Recent developments in the design of oral antigen formulations was discussed in this review [135-137].

Author details

Hyunjo Kim

Address all correspondence to: hyunjokim@hotmail.com

Pharmacy School of Sahmyook University, Seoul, Korea
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