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REVIEW ARTICLE

Design of Nanoparticles for Colon Target Drug Delivery – A Review

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ABSTRACT

Targeting of drugs to specific sites of action provides several advantages over non-targeted drugs. Colon specific drug delivery had gained increased importance just for the delivery of drugs for the treatment of local diseases associated with the colon. For the successful targeting of drugs to the colon, the drug needs to be protected from degradation, release and or absorption in the upper portion of GI tract and then to ensure the abrupt or controlled release in the proximal colon. General approaches for colon targeting drug delivery includes use of prodrugs, pH dependent system, time dependent systems and colonic microflora activated systems. Multifunctional nanoparticles play a significant role in cancer drug delivery. Nanoparticles exploit biological pathway to achieve payload delivery to cellular and intracellular targets. The targeting schemes explored for many of the reported nanoparticles systems suggest the great potential of targeted delivery to revolutionize cancer treatment. This review article deals with anatomy and physiology of colon, development of colon cancer, various approaches for colon targeting and evaluation of drug release in colon to give a updated information for the need and development of drug loaded nanoparticles for colon cancer.

Keywords: Colon, Nanoparticles, Targeted drug delivery.

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INTRODUCTION

Human body is made up of hundreds of millions of living cells. Cancer begin when cells in a part of the body starts to grow out of control. There are many kinds of cancer, but they all starts because of out-of control-growth of normal cells to abnormal cells. In most cases the cancer cells form a tumor [1]. Colon cancer is an unregulated growth of cells within the tissue of the colon. These cancerous or malignant cells grow and multiply to create a mass of cancerous tissue called a tumor. It invades and destroys normal adjacent tissues. Radiations and pharmacological therapy play an increasingly important role at later stages of diseases both as adjuvant to surgery and in reduction of symptoms [2].

The chemotherapeutic treatment of colon cancer has undergone revolution in the past 5-10 years with a variety of new drugs and regimens being either approved or under investigations. In the area of targeted delivery, the colonic region of GI tract is the one that has been embraced by scientists and is being extensively investigated over the past 2 decades [3]. Targeting of drugs to specific sites of action provides several advantages over non-targeted drugs. A drugs needs to be protected from degradation, release and or absorption in the upper portion of GI tract and then to ensure abrupt or controlled release in the proximal colon while targeting drugs to colon [4].

Nanotechnology has achieved the status as one of the vital research endeavors of 21st century, which may be called as “Nano-Century” with nanotechnology making its presence felt in different spheres of lives [5]. Nanoparticles are at the cutting edge of the rapidly developing area of nanotechnology. Nanoparticles are small colloidal particles that are made non biodegradable and biodegradable polymers, and their diameter is around 200 nm [6]. The drug is dissolved, entrapped, encapsulated or attached to nanoparticles matrics [7]. It may also offer a plenty of advantages over conventional dosage forms, which include improved efficacy, reduced toxicity, enhanced biodistribution and improved patient compliance [5]. In this review article, the anatomy and physiology of colon, pathophysiology, screening methods have been discussed for the better understanding of colon cancer. Additionally, various approaches for colon targeting and evaluation of drug release are discussed for the necessity of drug loaded nanoparticles for colon cancer. This article will help the persons involved in the research of colon target drug delivery for colon cancer.

EPIDEMIOLOGY

Colon cancer is the third most commonly diagnosed cancer and the third leading cause of cancer death. Overall, the lifetime risk of developing colon cancer is about 1 in 20. This risk is slightly lower in women than in men. The number of men and women affected by the colon cancer in India is about 610618 and 570793 in thousands respectively. The death rate from colon cancer has been dropping in men and women for more than 20 years [8, 9].

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COLON ANATOMY AND PHYSIOLOGICAL CONSIDERATIONS

The first and longest part of large intestine is colon, a muscular tube about 5 feet long. Water and minerals nutrients are absorbed form the food matter in the colon.
The colon had 4 sections:

1. The first section is called as the 'Ascending Colon'. It starts where the small intestine attaches to the colon and extends upwards on the right side of the abdomen.
2. The second section is called as the 'Transverse Colon'. Since it crosses the body from the right to the left side in the upper abdomen.
3. The third section is the 'Descending Colon'. It continues downwards on left colon.
4. The fourth and last section is known as 'Sigmoid Colon' because of its "S" or Sigmoid shape [8]. [Figure 1]

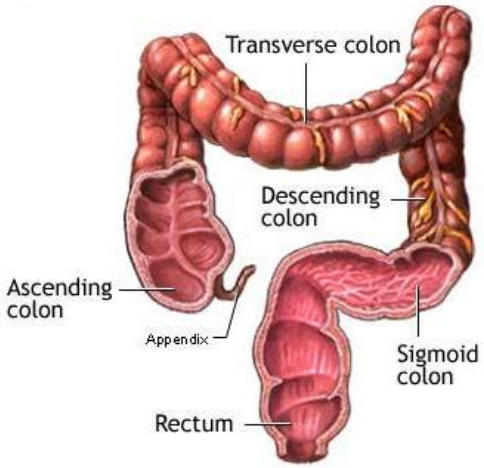

Figure 1: Structure of Colon

The physiological factors governing the colon drug delivery are gastrointestinal transit, small intestine transit, colonic transit, Gastric emptying, Stomach and intestinal pH, Colonic Microflora and Enzymes, Colonic absorption. It is must to go through the above considerations for the design of colon target drug delivery [10].

ABNORMAL GROWTH IN THE COLON [9]

Generally, the colon cancer develops slowly over several years. Before a cancer develop, a growth of tissue or tumor usually begins as a non-cancerous polyp on the inner lining of the colon. A tumor is abnormal tissue and can be benign (Not cancer) or malignant (Cancer). A polyp is a benign, non-cancerous tumor. Some polyps can change into cancer but not all do. The chance of changing into a caner depends upon the kind of polyp.

1. **Adenomatous polyps (Adenomas)** are polyps that can change into cancer. Because of this, adenomas are called pre-cancerous conditions.
2. **Hyperplastic polyps and inflammatory polyps,** in general are not pre-cancerous.

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SIGNS AND SYMPTOMS OF COLON CANCER [9]

Colon cancer may cause one or more of the symptoms below:

1. A change in bowel habits, such as diarrhea, constipation or narrowing of the stool, which lasts for more than few days.
2. Rectal bleeding, dark stools or blood in the stool.
3. Cramping or abdominal (belly) pain
4. Weakness and fatigue
5. Unintended weight loss.

PATHOPHYSIOLOGY [2]

The clinical and pathologic stages may be different in some cases. The stages describe the extent of cancer in the body. The various stages of a cancer are one of the most important factors in determining prognosis and treatment. Staging is the process of finding out how far a cancer has been spread. [Figure 2]

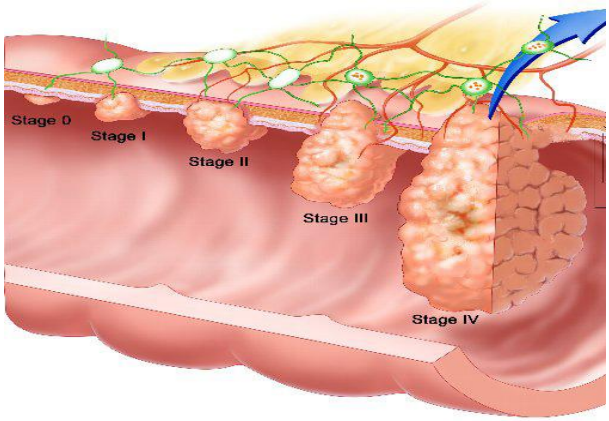

Figure 2: Various stages of colon cancer

Stage 0: Cancer has not grown beyond the inner lining of the colon
Stage 1: Cancer has grown through several layers of colon
Stage 2: Cancer has grown into the wall of the colon and may have extended into nearly tissue.
Stage 3: Cancer has spread to nearby lymph nodes but not to other parts of body.
Stage 4: Cancer has spread to distant organs and tissues.

SCREENING TEST FOR COLON CANCER [11, 12]

Screening is the process of looking for cancer in people who have no symptoms of the diseases. Several different tests can be used to screen for colon cancer. These tests can be divided in to 2 broad groups.

1. Tests that can find both colon polyps and cancer
2. Test that mainly find cancer.

Test that can find both colon polyps and cancer

Flexible sigmoidoscopy:

During this test, a sigmoidoscope a flexible, lighted tube about thickness of a finger with a small video camera on the end is inserted through the rectum and into the lower part of the colon to look at the part of the colon. Images from the scope are viewed abnormalities on a display monitor.

Colonoscopy:

Colonoscope, which is basically a longer version of a sigmoidoscope is inserted through the rectum into the colon to look at the entire length of the colon. The colonoscope has video camera on the end that is connected to a display monitor.

Double – contrast barium enema:

The double contrast barium enema (DCBE) is also called as air-contrast barium enema or a barium enema with air contrast. It may also be referred to as a lower GI series. It is basically a type of X-ray test. Barium sulfate, which is a chalky liquid and air are used to outline the inner part of the colon to look for abnormal areas on X-rays.

CT Colonography (Virtual colonoscopy):

This test is an advanced type of computed tomography (CT or CAT) scan of the colon. A CT scan is an X-ray test that produces detailed cross-sectional images of the body. CT colongraphy has a special computer program which creates both 2-dimensional x-ray pictures and a 3-dimensional “fly-through” view of the inside of the colon, which allows looking for polyps or cancer.

Tests that mainly find colon cancer [11]

Fecal occult blood Test:

The fecal occult blood test (FOBT) is used to find occult blood (blood that can't be seen with naked eye) in feces. The FOBT detects blood in the stool through a chemical reaction. If this test is positive, a colonscopy is needed to find the cause of bleeding.

Fecal immunochemical Test:

The fecal immunochemical test (FIT) also called an immunochemical fecal occult blood test (iFOBT) is a newer kind of test that also detects occult (hidden) blood in the stool. This test reacts to part of the human hemoglobin protein, which is found in red blood cells.

Stool DNA Test:

Instead of looking for blood in the stool, these tests look for certain abnormal section of DNA (genetic materials) from cancer or polyp cells. Cells from colon cancer or polyps with these mutations are often shed in the stool, where test may be able to detect them.

Tests to look for colon polyps and cancer

If symptoms or the results of the physical exam of the blood test suggest that colon cancer might be present. The following test must be done:

1. Biopsy
2. Imaging Tests.
    a. Computer Tomography (CT/CAT) Scan
    b. Ultrasound
    c. Magnetic Resonance imaging (MRI) Scan
    d. Positron Emission Tomography (PET) Scan
    e. Angiography

TREATMENT FOR COLON CANCER

The successful targeted delivery of drugs to the colon via the gastrointestinal (GI) tract requires the protection of a drug from degradation and being released in stomach and small intestine. This might be achieved by the use of special drug delivery system that can protect the drug during its transfer to the colon [13].

Advantages of colon drug delivery system over conventional drug delivery

Chronic colitis, namely ulcerative colitis and crohn's diseases are currently treated with anti-inflammatory agents [14]. Administration of drugs by oral and intravenous route produces systemic side effects including adenosuppression, immunosuppression, cushinoid symptoms and bone resorption [15]. Thus selective delivery of drugs to the colon could not only lower the required dose but also reduce the systemic side effects causes by high doses [16].

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COLON TARGETED DRUG DELIVERY

Targeting delivery drugs to the colon has attracted much interest recently for local treatment for a variety of colonic diseases, such as irritable bowel syndrome (IBS), Colon cancer and inflammatory bowel diseases (IDB), which includes both ulcerative colitis and Crohn's diseases. Targeting of drugs to specific sites of action provides several advantages over non – targeting of drugs such as the prevention of side effects of drugs on healthy tissues and reduction of doses [17].

Furthermore, the treatment of colon diseases such as Ulcerative colitis, Colon cancer and Crohn's diseases is more effective with direct delivery of drugs to the affected area [18].

Several approaches have been developed for targeting colon drug delivery. Most of them utilize the following four main properties of GI tract and colon.

i) Approximation of transit time of small intestine
ii) Different physiological conditions in different branches of GI tract
iii) Specificity of bacterial enzyme localized in the colon
iv) Targeting of special drug delivery system to colon utilizing targeting moieties specific to colon [19].

APPROACHES FOR COLON TARGET DRUG DELIVERY

A variety of approaches have been used and various systems have been developed for achieving colon targeting drug delivery [20].

These include:

a) Systems developed with pH sensitive polymers
b) Time – dependent formulations
c) Enzyme controlled release systems
d) Pressure dependent systems.

pH dependent approach:

This approach is based on the pH dependent release the drugs from the system. In this case, the pH difference between the upper and terminal part of GI tract exploits the effective delivery of drugs to the colon [3]. The pH is about 6.5 in the proximal small intestine and about 7.5 in the distal small intestine [21]. The pH in the terminal ileum and colon (Except ascending colon) is higher than in any other region of GI tract. Thus, a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region [22]. However, pH values of 5.7 have been measured in ascending colon and the pH is transverse colon is 6.6 and 7.0 in the descending colon. This system effectively resists drug release under acidic conditions of the stomach. A considerable amount of drug may be released in the small

intestine before it reaches the colon [23]. Use of pH dependent polymers is based on these differences in pH levels. The polymers used for colon targeting, should be able to withstand the lower pH values of stomach and the proximal part of small intestine and must be able to disintegrate at the slightly alkaline pH. These processes distribute the drug throughout the large intestine and improve the potential of colon targeting delivery systems [22]. Eudragit, more specifically Eudragit L & S are the principle group of polymers utilized for the preparation of colon target dosage forms. Eudragit™ S coatings protects the drug release in the upper parts of the gastrointestinal and have been used in the preparation for colon-specific formulations [24].

***Time dependent approach*:**

The time dependent approach is also known as pulsatile release, delayed or sigmoidal release system. Usually, time dependent drug delivery systems are designed to deliver drugs after a lag of five to six hours. The lag time depends upon the size of the dosage form and gastric motility associated with the pathological conditions of the individuals [3]. Time controlled formulations for colonic delivery are also delayed release formulations to which the delay in delivery of the drug is time based [25,26]. The time dependent formulation for colonic delivery contains a pH dependent coating component because the transit of a formulation in the GI tract is largely influenced by the gastric emptying time [27]. This coating is also used to prevent rapid swelling and disintegration in upper GI tract since other controlled release components based on the mechanism of swelling, osmosis or a combination of the two are often included in the time dependent release formulation [17,23].

Enzyme controlled systems:

Among all the approaches used for the colon targeting the microbial controlled system is the most preferable and promising because of its unique enzymatic ability of the colonic microflora. It enables a more specific targeting, independent of pH variations in the GI tract [28]. The colonic bacteria are predominantly anerobic in nature and secrete enzyme that are capable of metabolizing substrates such as carbohydrates and proteins that escapes the digestion in the upper GI tract [29].

The enzymes present in colon are:

1. **Reducing enzymes:** Nitroreductase, Azoreductase, N-oxide reductase, sulfoxide reductase, Hydrogenase etc.,
2. **Hydrolytic enzymes:** Esterases, Amidases, Glycosidases, Glucoronidase sulfatase etc.,

Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site specific approach as compared to other approaches. These polymers shield the drug from the environments of stomach and small intestine and are able to deliver the drug to the colon on reaching the colon. They undergo assimilation by microorganism or degradation of enzyme or

break down of polymer leading to a subsequent reduction in their molecular weight. This can be achieved by targeting prodrug delivery like 1) Targeting specific enzyme, 2) Targeting specific membrane transporters and 3) Polysaccharides based systems [30].

Pressure dependent drug delivery

GI pressure is another mechanism that is utilized to initiate the release of drugs in the distal part of the gut [3]. Viscosity of the luminal contents within the colon is greater than at other sites within the GI tract due to the reabsorption of water form large intestine. This change in viscosity leads to an increase in pressure resulting from the peristaltic forces. This pressure change can be used to trigger drug release. For example, pressure controlled colon delivery capsules made up of ethyl cellulose once taken orally they behave like an ethyl cellulose balloon. The reabsorption of water in the colon causes the viscosity of the luminal content to increase. As a result, the increased intestinal pressure directly affects the system via colonic peristalsis. In response to the raised pressure, there is a rupture in the capsule and the drug is released in the colon [31,32].

NANOPARTICLES IN DRUG DELIVERY

Nanoparticles are the versatile drug delivery system that can overcome physiological barriers and target drugs to the specific site. Nanoparticles can be used therapeutically as drug carriers, either by dissolving, entrapping or encapsulating the active substance (drug or biologically active materials) or by adsorbing or attaching the active substances. Nanoparticles delivery systems may offer plenty of advantages over conventional dosage form, which includes improved efficacy, reduced toxicity, enhanced biodistribution and improved patient compliance. Nanoparticles based drug delivery systems have been used as a physical approach to alter and improve the pharmacokinetics and pharmacodynamics properties of various types of drug molecules. They are able to show controlled release properties due to their biodegradability, pH, ion and temperature sensibility [7]. The drug release rate can be affected by manipulating the degradation of the polymers [33]. A variety of methods have been used to prepare nanoparticles, those methods includes solvent evaporation, nanoprecipitation and multiple emulsion [34]. The use of biodegradable polymers for nanoparticles preparation was preferable for this application to prevent complications with long-term deposition of nanoparticles or any residual component inside the ulcerated tissue [35].

For colonic pathologies, it was shown that nanoparticles tend to accumulate at the site of inflammation in Inflammatory Bowel Disease (IBD). This is because in case of colitis, a strong cellular immune response occurs in the inflamed region [36]. A previous study proved that an increased nanoparticles deposition in the inflamed tissue of the colon compared to the healthy control [37]. Moreover, the degradation of dosage may cause the release of entrapped drug leading to systemic drug absorption and side effects. In order to overcome this problem, drug loaded nanoparticles are entrapped into pH sensitive microspheres, which serves to deliver the incorporated nanoparticles to their site of action, thereby preventing an early drug leakage. The

use of Eudragit S prevents the drug release in the upper GI tract and during intestinal passage and permitted selective drug delivery in the colon.

EVALUATION OF COLON TARGET DRUG DELIVERY

In vitro and *in vivo* methods are used to study the drug release behavior of the colon drug delivery system because it is necessary to conform that the dosage form remains intact in the physiological environment of the stomach and small intestine and should be releasing the drug only in the colon.

In vitro studies

The ability of the carrier to remain unaltered in the physiological environment of the stomach and small intestine is generally assessed by conducting drug release studies in pH 1.2, 5.0 and 7.4 for 24 hours using USP dissolution test apparatus [38]. Dissolution testing is probably the most widely used methodology for evaluating oral modified release delivery systems including those for colon-specific drug delivery. This necessitates that the dissolution method be discriminative, reproducible, scientifically justifiable and more importantly biorelevant [28]. The ability of the delivery system to release the drug in the colon is tested *in vitro* by estimating the amount of drug released at different time intervals, by finding out the degradation of the carrier [39].

In vivo/Pharmacokinetic studies

In vivo studies are usually conducted to evaluate the site specificity of drug release and to obtain relevant pharmacokinetics information of the delivery system. Different animals have been used to evaluate the performance of colon specific drug delivery systems such as rats, pigs and dogs which closely simulate the human physiological environment of the colon. The selection of an appropriate animal model for evaluating a colon-specific delivery system depends on its triggering mechanism and system design [40]. The following techniques are used for monitoring the *in vivo* behavior of colon specific delivery systems like 1) Gamma Scintigraphy and 2) Roentgenography.

LIMITATIONS AND CHALLENGES [3,29]

1. Establishing an appropriate dissolution method in designing *in vitro* system is one of the challenges in developing a colon specific drug delivery. As a site for delivery of drugs offers a near neutral pH, reduced digestive enzymes activity, a long transit time and increased responsiveness to absorption enhancers. Hence, targeting is complicated with reliability and delivery efficiency [41].
2. Limiting factors for poorly soluble drug as the fluid contents in colon is much, lower and it is more viscous than in upper part of GI tract. For successful delivery through this site,

the drugs require to be in solution form before it arrives to colon or it should dissolve in luminal fluid of colon.

3. The resident microflora could also affect colonic performances via metabolic degradation of drug.
4. There is a wide range of pH values and different enzyme present throughout the GI tract, through which the dosage form has to travel before reaching the targeting site, further complicating the reliability and delivery efficiency.
5. Lower surface area and relative "tightness" of the tight junction in the colon can also restrict drug transport across the mucous and into the systemic circulation [42].

CONCLUSION

The importance of colon target drug delivery is that the drug released from the system must be sensitively active in the colon. Drug targeting to the diseased colon are advantageous in reducing the systemic side effects, lowering dose of the drug and supplying the drug only when it is required and maintaining the drug as possible for colon targeted drug delivery. There is a need to develop a novel approach which is specific for colon targeting. Colon specificity is more likely to be achieved with systems that utilize natural materials that are degraded by colonic bacterial enzymes. The challenge remains to develop and validate a dissolution method that incorporates the physiological features of the colon. In future, various multifunctional novel nanoparticles based drug delivery may be designed and developed for the treating the colon cancer.

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