Can curcumin along with chemotherapeutic drug and lipid provide an effective treatment of metastatic colon cancer and alter multidrug resistance?

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A B S T R A C T

Cancer is one of the most deadly diseases spreading all over the world and a major cause of fear in the society. Colon cancer is the 4th most common cancer causing death in both male and female equally, mainly caused due to the improper diet plans, consumption of the red meat and lack of exercise. Although the design of the chemotherapeutic drugs is well advanced, many of them developed resistance towards the cancer cells. The major reason behind the drug resistance in the colon cancer cells is due to the action exhibited by P-gp, which belongs to a member of ABC transporter family. P-glycoprotein (P-gp) effluxes the drug from its entry into the cancer cells, by treating it as a foreign body and hence decreases the therapeutic concentration of chemotherapeutic drugs inside the cancer cells. For overcoming this scenario, we posit the use of the curcumin (as a flavonoid) along with the lipid and the chemotherapeutic drug to provide an effective therapy and to overcome the possible issues associated with the failure in the therapy. Curcumin possesses dual mode of actions as a chemosensitizing agent and also as a chemotherapeutic drug. It generally acts as a chemosensitizer which can alter or inhibit the efflux pump exhibited by P-gp and provide a pathway for the entry of the chemotherapeutic drug into the cancer cells. Lipids have the potential to overcome the Multidrug resistance (MDR) and related issues; in addition, lipids are used for targeting colon cancer cells and also can act during the metastatic condition of the cancer which is hypothesised to be proven by using various studies. If our hypothesis is proven, the use of curcumin with lipids and the chemotherapeutic drug in a novel combination will reduces the majority of the issues related to the multidrug resistance, the recurrence and the spread of cancer could be overcome in a safe and effective manner.

Introduction

Cancer commonly known as malignancy is the abnormal growth of the cells in our body. Breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, along with more than 100 types of cancer is mostly discovered until this era. Symptoms that they exhibit depend upon on their types and origin. Colorectal cancer is also known as colon cancer, rectal cancer or bowel cancer is any kind of growth, lump or a tumor in the colorectal region [1]. Colorectal cancer is the second biggest cancer killer globally. Centre for development studies (CDS) and the World Health Organisation (WHO) reported that colorectal cancer is the third-most cancer worldwide after lung cancer, with nearly 1.4 million new cases diagnosed by 2012. This number is expected to be increased to 24 million by 2035. American cancer society surveyed that 1 in 20 people in the US developed colorectal cancer in their lifespan. Colorectal cancer influences both men and women equally, but men favour to develop this in their younger ages itself. The causes leading to the development of colorectal cancer is not well known but experts say that diet and hierarchy plays the major role in it. The stages of colon cancer are determined mainly based on the invading of the cancer cells to the different layers. Metastasis that is the spread of the cancer cells to other parts of the body mainly occurs in the 4th stage or the Duke E stage is mainly due to the spread of the cancer cells through the lymph node, which is also the predetermined cause for the recurrence of the disease after the treatment.

The different treatment methods of colorectal cancer including surgery, chemotherapy and radiotherapy mainly depend on the factors, current stage, and the size of the tumor and even the overall health of the patient. Surgery is the most common treatment for colorectal cancer if the cancer is diagnosed in the early stage itself. Surgery will not cure the disease; it can only ease the symptoms. Colectomy is mainly done to remove the part of the colon with cancer but it may lead to major blood loss, clots and infections. The patients even have to undergo severe pain, swelling and unexpected emotional or physical stress reactions due to the treatment methods. Radiotherapy is another method of treatment where it uses higher radiation energy to destroy the cancerous cells, but in some patients, it can develop rectal irritation, painful bowel movement or blood in the stool.

Chemotherapy involves using chemicals or drug substances to destroy abnormal cells. It is used mainly before and after the surgery as it...
helps to shrink the tumour cells and can lower the chances of recurrence. Commonly used medication for colorectal cancer includes 5-fluorouracil (5-FU), oxaliplatin, irinotecan, cetuximab, bevacizumab, panitumumab, capcitabine, regorafenib etc. Chemotherapy mostly kills the drug-sensitive cells leaving behind the drug-resistant ones, when the tumor cells begin to grow again and again then the chemotherapy fails as the cells becomes resistant to the current medication. Multidrug resistance caused by the cancer cells to a broad spectrum of anticancer drugs is another cause for the failure in the chemotherapy. Over expression of P-glycoprotein prevents the influx of chemotherapy drugs to the cancer cells and creates an obstacle during the therapy, finally leading to multidrug resistance. Mostly 50% of the drugs now became resistant to the cancer cells; hence a new strategy is in need to be developed to overcome the resistance caused by the cancer cells and for the success of the therapy [2].

Flavonoids are the most prevailing polyphenolic compound found in our human diet and are the secondary metabolite found in both plants and animals. Its activity as an antineoplastic agent and its action as a natural P-gp inhibitor were reported in various surveys and different literatures. Since they have anticancer activity, in combination with 5-fluorouracil it is hypothesized to produce a synergistic effect in our body [3]. The multi-use of the lipid adds on several advantages like improved bioavailability, targeting of the drug to the tumor cells, lymphatic targeting of the drug, and in turn, having an additional advantage over the metastatic stage with a reduced toxicity.

The main challenge faced in colorectal cancer therapy is Multidrug resistance (MDR). P-gp is a major cause of multidrug resistance in cancer cells [4]. Multidrug resistance (MDR) is a phenomenon where the cancer cells efflux the drug and other foreign bodies which ultimately results in the reduced concentration of the drug inside the cells which leads to inefficacy during the therapy [5].

P-gp leads a major role in regulating the distribution and bioavailability of drugs, where its expression in the intestine and other parts of the gastrointestinal tract reduces the absorption of drug and its substrate. Hence bioavailability and therapeutic plasma concentration of the drug is not attained to the level. But in turn, if the P-gp expression is reduced, the drug will reach to attain the therapeutic plasma concentration [6]. The substrate enters into P-gp through an opening at the cytoplasmic side of protein or through an opening in the inner leaflet of the membrane. ATP (Adenosine triphosphate) will bind at the cytoplasmic side of the protein. ATP hydrolysis alters the substrate which is to be removed from the cell. When phosphate is released from the native ATP molecule, the substrate will get excreted. A new molecule of ATP binds to the secondary ATP binding site when Adenosine diphosphate (ADP) is released. This process will restart again when the hydrolysis and discharge of ADP and a phosphate molecule reboot the protein [7]. The activity of the P-gp can be reversed by using flavonoids.

Flavonoids are used in our day to day life. Flavonoids remain in the digestive system and get absorbed by the cells in the bowel. Various studies revealed that flavonoids kill cancer cells and prevent them from further multiplication. The mechanism of their inhibition is by targeting certain biomolecules such as enzymes, DNA and proteins [8,9]. The flavonoids are used as a natural inhibitors and as a modulator in the action of the P-gp which have more potential effect in case of safety, efficacy and economical when compared with the chemical or synthetic inhibitors [10]. Most of the drugs are having limitations in anticancer treatment due to multidrug resistance which can be defeated by the synergistic action of flavonoids.

Curcumin as a flavonoid has an additional advantage as it can reverse MDR caused by P-gp [10]. Reports revealed that curcumin has the ability to inhibited P-gp by acting on P13K/Akt/NF-kB [11] pathway in MDR leukemia L1210 cells induced in the mouse. Choi et al in 2008 proved that when Adriamycin and curcumin are given in combination, the results obtained from western blotting clearly shown that it can cleavage the poly-ADP ribose polymerase (PARP) inhibitors and can overcome MDR caused by P-gp [12]. The anticancer properties of curcumin had already proven by using cultured cells and animal studies. Curcumin inhibits lipoxigenase activity and acts as a specific inhibitor of cyclooxygenase-2 expression. It inhibits the initiation of carcinogenesis by inhibiting the cytochrome P-450 enzyme activity and increasing the levels of glutathione-S-transferase. It suppresses the promotion/progression stages of carcinogenesis and even alters the growth of DNA mismatch repair defective colon cancer cells. Therefore, curcumin has the value as a safe chemotherapeutic agent for the treatment of tumors exhibiting DNA mismatch repair deficient and microsatellite unstable phenotype [13].

Lipids have various deals for the treatment of colon cancer. Lipids can be used for the target specific action of the drug to the colon cancer cells, to alter the MDR related issues and during the metastatic stage of cancer. Since the metastasis of the cancer cells mainly originates through the lymph nodes this approach may have the potential to treat metastasis malignancy as the transport of the lipids mainly take place through the lymph nodes [14–18]. For the rapid proliferation, cancer cells utilize and metabolize a high quantity of lipids compared to that of the normal ones. They mostly utilize the fatty acids as a source of energy and as a precursor for various biological processes. This is meant to be a kind of stealth mechanism where the lipids are considered as an energy source to the cancer cells which is helpful for targeting the drug specifically to the cancer cells thereby the degree of toxicity to the normal one could be reduced. This lipophilic pro-drug approach releases the lipids by cleavage, leaving behind the parent drug then gets eventually distributed into the tumor cells to elicite its cytotoxic effect.

The schematic representation of the mechanism of action of the conjugates is discussed in Fig. 1. When one of the fatty acid is replaced with the drug molecule then the drug can enter into the triglycerides (TG) deacylation-reacylation pathway. TG hydrolyses to 2-monoglyceride (2-MG) and free fatty acid in the lumen of the gastrointestinal tract, where the monoglyceride get absorbed into enterocytes and will get reacylated to form TG. TG will then be incorporated into lipoprotein, followed by its accumulation into the lymphatic system. This drug conjugates will utilize lymphatic transport for its lymphatic drug targeting and enhance drug absorption [14,17,19]. The function of the lymphatic system for the transport of dietary lipids from the intestine to the lymphatic capillaries facilitates the lipid drug combination to efficiently use this pathway and to integrate into the enterocytes and enter into the lymphatic capillaries and hence to overcome the first pass metabolism exhibited during the oral administration of the drug [16]. The schematic representation of the fate of lipids in our body is represented in Fig. 2.

The drugs of choice used in our hypothesis are 5-Fluorouracil and curcumin. 5-Fluorouracil is hydrophilic in nature whereas curcumin is hydrophobic in nature. The conjugation of these two drugs is practically impossible. Lipids are mostly used to create a link or bond between the drugs. The other major issue associated with both 5-Fluorouracil and curcumin is with its poor bioavailability, metabolism and elimination. Bioavailability of 5-Fluorouracil is only around10-20%. These issues can also be solved by conjugation with the lipids.

**Hypothesis and prediction**

The major challenge faced during chemotherapy is the multidrug resistance caused by chemotherapeutic drugs and the metastasis of the cancer cells to other parts of the body (mainly the liver). Many of the chemotherapeutic drugs are designed for the treatment of colon cancer, but most of them attained resistance to the cancer cells [1]. They are associated with the other problems such as its side effects, low sensitivity and drug-drug interaction. Multidrug resistance in colon cells is mainly associated with the efflux pump mechanism exhibited by the P-gp. To inhibit the P-gp and to provide a proper channel for the drug to enter into the cancer cells, lipid and curcumin are used [20]. In this proposal, we hypothesized to use 5-Fluorouracil which is used as the
first line therapy for the treatment of colon cancer from various decades which attained resistance to the colon cancer cells, curcumin as a chemosensitizer and as a chemotherapeutic agent, lipids as an inhibitor for reversing MDR and related issues for targeting colon cancer cells and could be used during the metastatic stage since the lipids are transported through the lymphatic system and the metastasis of the cancer cells originate through the lymph nodes.

Usually, chemosensitizers have the ability to bind with the P-gp transporter domain but curcumin as a flavonoid possess a dual function in reversing MDR and also as a chemotherapeutic agent. Lipids also possess a dual mode of action; lipids have shown with their ability to overcome the MDR related issues and increase in the efficacy of the chemotherapeutic drugs, the utilization of the lipids as an energy source for the growth and the development of the colon cancer cells and hence can be used for its targeting [4]. The transport of the lipids through the lymphatic system helps during the treatment of the metastatic condition since the spread of the cancer cells occurs through the lymph nodes. If our hypothesis is proven to be true, the use of curcumin with lipids and the chemotherapeutic drug as a novel combination will reduce the major issues related to the multidrug resistance, the recurrence and the spread of cancer could be overcome in a safe and effective manner. The preparation of curcumin lipid 5-Fluorouracil conjugates helps to develop a novel technique for the treatment of colon cancer. Lipids creates a platform for both 5-Fluorouracil and curcumin coming

![Schematic representation summarising the mechanism of the 5-FU lipid curcumin conjugates for the treatment of colon cancer.](image-url)
from hydrophilic and lipophilic background respectively to bind with each other and the lipid dual drug conjugate is used in our hypothesis to improve the bioavailability, plasma concentration and cellular permeation.

Validation and discussion

Validation of the above said hypothesis is experimentally feasible. The P-gp inhibitory activity could be found out by using the P-gp assay. The efflux mechanism involved in the drug resistance can be evaluated by using Multidrug resistance1-Madin Darby Canine Kidney (MDR1-MDCK), MDCK and Carcinoma cell line (Caco2). The relative comparisons of the cell lines are determined for obtaining a better result. Western blotting results also depict the inhibition of compound to the resistant part [21]. Researchers in 2011 examined and proven that the curcumin can differentiate between the normal cells and the cancer cells, it can actively impose apoptosis in cancer cells. The absorption of the curcumin in the colon cell lining, when compared to the other organs, add on the advantage for the treatment of colon cancer [11]. The main advantage of the use of the curcumin as a chemotherapeutic agent and the P-gp inhibitor is even during its use in a high dose, the flavonoids produce less or no toxic effect and it don’t produce any toxic effect to the normal cells.

As a part of the proposal a conjugate of curcumin, lipid and the chemotherapeutic drug conjugate could be made. The conjugate is prepared by using the enzymatic conjugation and facile esterification method. Lipid drug conjugates which contain a chemotherapeutic drug and a flavonoid can be used for the treatment of colon cancer in many aspects. Lipid drug conjugates (LDC) is developed by the method of conjugation of the lipid with the parent drug to form a covalent or non-covalent bond. The conjugation of the drug with the lipid increases the lipophilicity of the drug [7]. The effective delivery of the drug to the target site could be made possible by using the lipid drug conjugate. The use of the lipids is in the form of fatty acid here. The cleavage and the absorption of the drug in the cancer cells could be studied with the help of in vitro studies. For this, the in vitro lipolysis medium is used. The calcium and bile salt present in this media help in digestion of the lipids (lipolysis) [22]. The release of the drug by dissociation of the bond can be studied by using this method. The lipids are readily used by the cancer cells for their growth and development. Presence of the specific receptors for lipids on the membrane of the cancer cells recognizes the lipids as a source of energy, where the cancer cells use...
them for their growth and the development.

Lipid compounds enter into the lymphatic system by initiating the production of chylomicrons. It enters into the lymphatic system by activating the triglyceride core of the chylomicrons. To evaluate the fate of absorption and circulation of the lipids through the lymphatic system could be studied by using the intestinal lymph node cannulated mouse model [23]. This model is useful to find out the uptake and the lymphatic transport of the lipids including fatty acids and cholesterol. The same could be evaluated by ex vivo experiments by identifying/locating and then isolating lymph nodes and extracting the drug for quantifying the drug concentration in the lymphatic system. The lipid absorption study could be found out by using the cell culture model, such as CaCo-2. The use of this model is potentially useful with the ability of the CaCo-2 cell lines to produce chylomicrons. With the comparison and the correlation between the two models provides a clear report on the lipid absorption and circulation in the lymphatic system could be made [24]. Researches demonstrated the activity of the lipids playing a significant role in suppressing the MDR caused by P-gp tested in vitro using the ovarian cancer cells. The method used for the evaluation of the action of the lipids on MDR cells or to produce an inhibitory action on cancer cells is studied by using the Protein expression assay, western blotting, immune-histochemistry and in vitro cell line studies.

The tumor markers or biomarkers are the substances which are produced by the cancer cells or the other cells of our body in response to cancer. The colorectal cancer biomarkers can be found in the blood which can be measured by using blood test or in the tumor tissues itself. The biomarker which is found in the blood, which may elevate in colorectal cancer, is carcinoembryonic antigen (CEA) and CA 19-9. The biomarkers found in the tumor tissues are Microsatellite instability (MSI) and K-RAS mutations [25]. The biomarkers level can be used to elevate the prognosis of cancer after diagnosis and for making treatment decisions [26]. From the level of the biomarkers, the response of the colon cancer cells towards our hypothesized combination could be evaluated. The metastasis with the cancer cells can be studied by using the cell migration assay. If the cancer cell is shown with no migration, then we can conclude the cell migration assay. If the cancer cell is shown with no migration, then we can conclude the cell migration assay. If the cancer cell is shown with no migration, then we can conclude the cell migration assay. If the cancer cell is shown with no migration, then we can conclude the cell migration assay.

With our hypotheses we address the following issues a) a novel method to overcome, and to altering the MDR by inhibiting the P-gp efflux pump b) Targeting of the conjugates in the lymphatic system to suppress metastatic stage and c) increasing the efficacy of the treatment. If our hypothesis is proved, it will provide an effective, safe and feasible therapy which could be used even during the metastasis stage of the colon cancer also. Our proposed formulation will even reduce the dosing frequency of the drug since the flavonoids are having the synergistic action in the cancer cells. This formulation may overcome the barriers for the failure of the treatment and may evolve with a new strategy for producing a cytotoxic effect in the cancer cells.

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Declaration of Competing Interest

The author reports no conflict of interest.

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Appendix A. Supplementary data

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References