REPURPOSING ALBENDAZOLE AND MEBENDAZOLE, ANTIPARASITIC DRUGS IN COLORECTAL CANCER TREATMENT- A REVIEW

By

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Declaration

It is hereby declared that.

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Repurposing Albendazole and Mebendazole, an Antiparasitic Drug in Treating Colorectal Cancer- A Review.” submitted by Shawon Mondal (19146068) of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) in February 2023.

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Ethics Statement

This study does not involve any kind of animal and human trial.

Abstract

Repurposing drugs may be the answer to overcoming these challenges associated with cancer treatment. Albendazole (ABZ) and Mebendazole (MBZ), which are often used to treat helminthic infections, have demonstrated significant promise as cancer treatments. However, neither their clinical importance nor their obvious mechanism in CRC treatment have been thoroughly examined. To learn more about these mechanisms and their clinical relevance, researchers have looked at the regulation of apoptosis in the human CRC cell lines HCT-15, HCT-116, HT-29, and SW480, selective apoptotic cell death in CRC adenocarcinoma cells in their G2/M phase, and DNA fragmentation mechanism through oxidative damage to CT-DNA by ABZ. The literature highlighting the potential anticancer mechanisms of MBZ, the inhibitory mechanism of tubulin polymer in CRC cell line, the inhibition of angiogenesis in exiting metastatic tumor, and the induction of the immune system via proinflammatory(M1) mediation and DYRK1B kinase modulation are also summarized in this review. To establish clinical relevance and highlight the therapeutic efficacy of ABZ and MBZ, this review also reviews the pertinent literature and clinical trials, in vivo, and in vitro investigations that are currently accessible.

Key words: Colorectal Cancer; Oxidative Damage; In Vivo, In Vitro, Metastatic Tumor

Dedication

Dedicated to the innocent lives that cancer has taken

Acknowledgement

First, I want to give thanks and appreciation to God, the Almighty, for granting me good health as well as the patience, dedication, and knowledge I needed to finish the thesis.

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List of Acronyms

ABZ	Albendazole
MBZ	Mebendazole
CRC	Colorectal Cancer
EMR	Endoscopic Mucosal Resection
TEM	Transanal Endoscopic Microsurgery
LAR	Low Anterior Resection
VEGFR	Vascular Endothelial Growth Factor Receptor
mCRC	Metastatic Colorectal Cancer
EGFR	Epidermal Growth Factor Receptor
MT	Microtubule
NAC	N-Acetyl Cystine
DYRK1B	Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1
NK	Natural Killer
MTX	Methotrexate
PLGA	Poly D, L Lactic Co Glycolic Acid
NP	Nanoparticle

Chapter 1

Introduction

1.1 What is cancer?

When body's cells regenerate in an uncontrollable manner and spread to other internal organs, it is called cancer. Cancer may appear almost anywhere in the trillions of cells that make up the human body. When the body needs new cells, human cells often divide and enlarge to produce them. This methodical procedure sometimes goes wrong, allowing damaged or abnormal cells to multiply when they shouldn't. From these cells, tissue lumps known as tumors may form. Cancerous tumors may move to remote regions of the body to generate new tumors. They can also infiltrate surrounding tissues. Leukemias and other blood cancers often do not result in solid tumors, although many other malignancies do. Noncancerous tumors cannot invade or spread to nearby tissues. After removal, benign tumors often don't return, although malignant ones sometimes may. However, benign tumors may sometimes develop into extraordinarily large masses. Some may have serious negative effects or even be deadly, such as benign brain tumors. Changes in the genes that control our cells' functioning, notably how they grow and divide, result in the genetic disease of cancer. Cell division errors, DNA damage from risky environmental variables including the chemicals in cigarette smoking, and sun's UV rays may all result in cancer-causing genetic mutations. Parents may pass on their cancer to their children. The human body often gets rid of DNA-damaged cells before they develop into cancer. But as we become older, the body becomes less capable of doing so. This contributes to the increased chance of developing cancer later in life. Lung, prostate, stomach, and liver cancers are more common in men, whereas breast, colorectal, lung, cervical, and thyroid cancers are more common in women (Cancer, n.d.).

1.2 Types of cancer

1.2.1 Based on layers of tissue affected

1.2.1.1 Carcinoma: The most common type of cancer is a carcinoma. Cells that line internal organs or the exterior surfaces of bodily components are where cancerous growths first appear. They often affect the breast, lungs, bladder, colon, and prostate along with other glands and organs that may secrete.

1.2.1.2 Lymphoma: Lymphomas are blood cancers that influence the immune system and are tumors of the lymphatic system. It interferes with the body's regular lymphocyte production process. The lymphocytes don't function correctly and tend to grow uncontrollably. Lymph nodes in particular places, such as the stomach, brain, intestines, etc., may be affected by lymphoma.

1.2.1.3 Myeloma: The bone marrow's plasma cells are the source of the blood malignancy known as myeloma. Because most patients have numerous bone lesions when it is detected, it is often termed multiple myeloma. White blood cells known as plasma cells may be seen in the bone marrow. They support the immune system's ability to combat infection. When malignant, these aberrant plasma cells invade the whole bone marrow, leaving insufficient room to produce healthy blood cells.

1.2.1.4 Sarcoma: Sarcoma originates in the fat, muscles, bones, cartilage, and connective and supporting tissues. One of the sarcomas known as osteosarcoma is bone cancer. The young are most typically affected. Sarcomas mimic the tissue in which they develop in appearance. For instance, mesothelial sarcoma or chondrosarcoma (of the cartilage), leiomyosarcoma (of the smooth muscles), rhabdomyosarcoma (of the skeletal muscles) (Cancer, n.d.).

1.2.1.5 Leukemia: Leukemia is a collection of malignancies that fall within the blood cancer umbrella. The bone marrow, which is the location of blood cell formation, is affected by several malignancies. When malignant, the bone marrow starts to overproduce immature white blood cells, which are unable to carry out their typical functions and often leave the patient vulnerable to infection.

1.2.2 Based on location

Depending on the location of malignancy cancer can be classified into broad groups like breast cancer, colorectal cancer, lung cancer, prostate cancer, blood cancer, bladder cancer, cervical cancer, prostate cancer etc (Carbone, 2020).

1.3 Clinical aspects and treatment options for colorectal cancer

Colorectal cancers (CRC) now considered among the third most diagnosed cancer worldwide (Daaboul & El-Sibai, 2018). CRC has now become one of the most common types of cancer affecting people of all ages and professions. CRC generally starts in the rectum and progress to the colon (large intestine). CRC occurs from colorectal polyps in the colon lining and transforms into cancerous polyp. Colonoscopy is one of the early-stage diagnosis method to detect colorectal polyp with the potential to develop cancer. Lifestyle choice plays a great role in the development of CRC. Occurrence and progression of CRC is associated with food habits as well. Reduced fibrous food intake and replacing them with high fat containing food subsequently increase the chance of CRC development. Lifestyle factors like smoking, lower physical activity, diabetes, alcohol consumption plays a great role in CRC progression. Most common type of CRC is adenocarcinoma. This CRC starts in the cell producing mucus in the colon and rectum area. There

are 5 distinct stages of colorectal cancer Depending on the invasiveness of the tumor (Daaboul & El-Sibai, 2018). Staging is a method used to specify the extent of cancer's invasiveness and can indicate recovery chances of the patient. Mostly CRC is diagnosed in an advanced stage, that's why proper diagnosis is crucial as the initial step in CRC treatment. CRC is associated with the formation of polyps in colon and rectal area, colonoscopy techniques are firsthand tools for the physicians to diagnose colorectal cancer. At first malignant polyps are identified through histopathological examinations, then these polyps are further classified into low risk and high-risk subcategories depending on the colonoscopic morphology and histopathological observations (Daaboul & El-Sibai, 2018). Differentiation between polyps is very crucial in the accurate assessment of degree of aggressiveness and malignant profile of them which helps in rational clinical decision making (Daaboul & El-Sibai, 2018).

1.4 Existing Medications based on stage

Choice of medication in CRC treatment and management depends on stage of cancer invasion and size and the shape of malignant polyps. Polyps less than 5 mm in diameter have a minimal risk of malignancy and are effectively handled with normal endoscopic net removal procedures, according to research. Local excision or endoscopic polypectomy should be performed on protruding polyps with favorable histological characteristics. Because of the high frequency of lymph node metastases, polyps with unfavorable histological characteristics should be surgically removed. During CRC treatment, drug of choice differs depending on the stage that the patient is in.

1.4.1 Existing treatment for Stage I patients

Stage I rectum adenocarcinomas are uncommon, they are typically treatable with surgical resection. Stage I CRC is considered as low risk metastasis and can be treated with endoscopic mucosal resection (EMR) (Daaboul & El-Sibai, 2018). The transanal endoscopic microsurgery (TEM) procedure is appropriate for small tumors. Low anterior resection (LAR) is an operating procedure used to surgically remove the cancerous polyps of upper rectal area. This procedure is done by removing cancerous polyps along with part of rectal tissue and lymph nodes (Daaboul & El-Sibai, 2018). Then, 5-fluorouracil (5-FU) or capecitabine, an acceptable adjuvant chemoradiotherapy can be prescribed.

1.4.2 Existing treatment for Stage II patients

In stage II CRC, the function of adjuvant chemotherapy is yet unknown. When treating CRC, 5-fluorouracil continues to be the core element chemotherapy (Daaboul & El-Sibai, 2018). Neoadjuvant chemotherapy and radiation therapy is recommended in the event of rectal cancer to reduce the tumor size before surgery. When the rectal tumor has migrated to nearby lymph nodes or has invaded the gut wall, radiotherapy is recommended. To make tumor cells more sensitive to radiation, 5-fluorouracil or capecitabine are often given along with radiotherapy (Daaboul & El-Sibai, 2018).

1.4.3 Existing treatment for Stage III patients

Stage III CRC is associated with the metastasis of lymph nodes in colon and rectal area. The recommended course of treatment at this point is a partial colectomy, which is the removal of cancerous polyp in the colon along with any nearby lymph nodes, followed by adjuvant chemotherapy (not more than eight weeks after surgery). But neo adjuvant and adjuvant therapies are recommended for the patients who are high risk of local recurrence of this cancer. Following a large surgical resection with anastomosis, oxaliplatin, 5-FU or folinic acid are recommended as the usual chemotherapy regimen. If oxaliplatin is contraindicated, 5-FU/LV given intravenously or oral fluoropyrimidines (capecitabine), are beneficial alternatives (Labianca et al., 2010). Other medications including the topoisomerase I inhibitor irinotecan, the anti-VEGFR medication bevacizumab. The usual chemotherapy to be given concurrently with radiation in neoadjuvant rectal treatment is still 5-fluorouracil (Daaboul & El-Sibai, 2018).

1.4.4 Existing treatment for Stage IV patients

Stage IV is the most dangerous since the cancer cells have metastasized to other organs, such as the liver, stomach, lymph nodes, lungs, or other organs. Currently, surgery is necessary to remove the colon and liver tumors. Part of the colon may need to be removed surgically also termed as partial colectomy. Sometimes resectable tumors are treated with chemotherapy along with radiation therapy, or chemotherapy alone may also be considered a viable treatment option. Systemic chemotherapy continues to be the major treatment strategy for unresectable malignancies and/or widely disseminated metastatic illness. All methods used to treat metastatic colorectal cancer (mCRC) primarily employ fluoropyrimidines (5-FU and capecitabine). Later, new

cytotoxic drugs such the topoisomerase I inhibitor (irinotecan) and the third-generation platinum analog (oxaliplatin) are also being used recently. Together with fluoropyrimidines, these drugs significantly advanced the therapeutic approach of mCRC. Along with several other novel medications now being studied, the combination of trifluridine, which is a nucleoside analog along with Tipiracil (thymidine phosphorylase inhibitor), has been authorized for the treatment of metastatic colorectal cancer (mCRC).

1.5 Drawbacks of existing treatment options

Diagnosis of colorectal cancer (CRC) is possible in advanced stage. Chemotherapy and targeted therapies very slightly increase the overall survival of these patients (van der Jeught et al., 2018). Resistance to therapy has a significant role in clinical outcomes. Escape strategies from both chemotherapy and targeted therapy continue to be the main offenders. Additionally, the efficacy of treating CRC with immune checkpoint inhibitors is limited by the low response rate to checkpoint inhibitors in patients with microsatellite instable high tumors (van der Jeught et al., 2018). Targeted therapy, adjuvant chemotherapy, surgery and neoadjuvant radiation therapy are the cornerstones of CRC treatment. However, each of these components has limitations that led researchers to look for safer and far more potent alternatives. There are certain negative aspects of chemotherapy, such as preexisting systemic toxicity, a suboptimal response rate, unexpected innate and acquired resistance, and poor tumor-specific selectivity (Xie et al., 2020). Drug resistance is still an issue given the dismal survival rates of CRC patients (van der Jeught et al., 2018). For instance, chemotherapy based on 5-fluorouracil (5-FU) continues to be the main treatment for CRC. Recently developed chemotherapy drugs include oxaliplatin, irinotecan, and

capecitabine. Advanced CRC is often treated with a combination of 5-FU, leucovorin, oxaliplatin, or irinotecan. The discovery of monoclonal antibodies like Bevacizumab and Cetuximab has considerably enhanced medical treatment for CRC. Even while response rates to various modulation approaches, including as chemotherapy and monoclonal antibodies have increased, the five-year survival rate for metastatic CRC (mCRC) is still just slightly over 12% (van der Jeught et al., 2018). One of the biggest obstacles to this observation is the rise of medication resistance. About half of patients with metastatic colorectal cancer (mCRC) who undergo 5-FU-based chemotherapy develop resistance to it (van der Jeught et al., 2018).

Monoclonal antibodies can directly control subsequent cell cycle progression and cell death. Furthermore, certain monoclonal antibodies target cells other than cancer cells, such immune cells, which aids in controlling the immune system's response to combat human cancer(Xie et al., 2020). Although monoclonal antibodies have many benefits and are now often used to treat CRC, individuals still experience substantial side effects from these medications. For instance, Cetuximab (Erbitux) is an antibody that hunts out the EGFR cell protein, which is present on healthy skin cells (as well as some types of cancer cells)(Xie et al., 2020). Some persons who use this medication may get severe rashes.

1.6 Drug repurposing in cancer treatment

Since the inception of drug development era for newer medications, drug development procedures and expenses related to it has been a major burden on finding newer therapeutic options for any certain disease. With the rising wases of newer form of disease and high demand for advanced therapeutic options, minimization and proper placement of fund and energy in a cost-effective way

has been one of the greatest concerns of drug developers. Cancer treatment options that are currently available in the market has taken up a huge load of resources in the past couple of decades, but the main concern is the rising number of newer forms of cancer and side effect and low selectivity profile of existing medication. In this dire situation drug repurposing has been proved to be a very reliable source of newer therapeutic option in cancer treatment. Colorectal cancer is among the most aggressive form of cancer which has been renowned to be metastatic, available treatment options like chemotherapy drugs, anticancer antibiotics, alkylating agents, or the combination of these has proved to contain many side effect profiles, lowers patients' quality of life, and proved to be harmful for healthy human cells. Keeping all these major concerns in mind preparing an already approved drug used as an antiparasitic solution like Albendazole could prove to be really advantageous in terms of less side effects and better selectivity.

1.7 Aim and Objectives

Regarding the anthelminthic drugs albendazole and mebendazole, we emphasize recent advances that have the clinical potential to improve responses to microtubule inhibitors in this review. We place particular emphasis on recent developments that have the clinical potential to increase the responsiveness of mebendazole and albendazole in the selective microtubule inhibition of CRC cancer cells with fewer side effects and improved treatment efficiency.

Our objectives include,

i. Encouraging researchers to focus on drug repurposing strategies and advancing research on the distinct anticancer mechanisms of ABZ and MBZ as well as their therapeutic potential.

ii. To pique researchers' interest in exploring ABZ and MBZ so they might improve CRC patients' quality of life by preventing the side effects of traditional anticancer therapies.

Chapter 2

Methodology

For this review study, relevant data were gathered by a comprehensive literature search. The material was collected from few reliable sources, including peer-reviewed papers and an online academic database. A thorough search of journals, review papers, research databases, clinical trial databases, and official websites were conducted to compile as much crucial information as possible concentrating on the repurposing of albendazole and mebendazole, two benzimidazole derivatives, in CRC therapy. Relevant data and articles were gathered while employing the finest of well-known and trustworthy data sources, such as Google Scholar, ScienceDirect, PubMed, and Scopus. The clinicaltrails.gov website was used to gather and update clinical trial data. Using the website biorender.com, figures and structures were generated. Based on relevant keywords, such as drug repurposing, the use of albendazole and mebendazole in the treatment of cancer, and the use of nanoparticles in the delivery of albendazole, relevant publications and data were gathered. Based on the title and keyword content, 109 articles have been evaluated. Then, after reviewing the abstracts, 78 articles were left. This review study was composed of 61 publications that were carefully chosen and analyzed. To respect the author's original works, correct and ethical referencing were performed using Mendeley software.

Chapter 3

Characteristics of Albendazole and Mebendazole

3.1 Albendazole and Mebendazole

It takes a long, expensive, and sometimes difficult procedure to find novel anticancer medications that are both more effective against tumors and less hazardous to healthy tissues, and these efforts frequently fail during the clinical trial stages required for drug approval. It's interesting to note that the repurposing of non-antitumor medications to be used in cancer therapy represents a valuable and alternative strategy, as candidate agents have well-documented pharmacokinetic and pharmacodynamic features, as well as good safety profiles, which may speed up their approval and implementation in the clinics. Benzimidazole anti-helminth medication with a broad range is Mebendazole (MBZ) and Albendazole (ABZ). These drugs are frequently given to treat a variety of parasitic worm infection in both domesticated animals and people. These medications are inexpensive and has little side effects. The anti-helminthics attach to the tubulin's colchicine-binding region and prevent microtubule polymerization, which reduces the absorption of glucose by helminth intestinal cells (Petersen & Baird, 2021a). Several recent in vitro studies have demonstrated the potential anti-cancer benefits of ABZ and MBZ (Petersen & Baird, 2021a). Microtubules in cancer cells are depolymerized and apoptotic nuclear morphology is induced by ABZ and MBZ. By encouraging microtubule depolymerization, ABZ and MBZ causes the cells to undergo apoptosis, which is characterized by condensed nuclei. Microtubules in cancer cells are depolymerized and apoptotic nuclear morphology is induced by ABZ and MBZ. By encouraging microtubule depolymerization, ABZ and MBZ causes the cells to undergo apoptosis, which is characterized by condensed nuclei. Albendazole (ABZ) and MBZ are regarded as a very efficient

therapeutic treatment for preventing tubulin polymerization in metastatic cells among microtubule (MT) targeting medications. A powerful inhibitor of tumor development, angiogenesis, and cell proliferation, ABZ and MBZ are benzimidazole derivatives that are typically used to disrupt the microtubule cytoskeleton.

3.2 The antiparasitic mechanism of Albendazole and Mebendazole

The microtubule networks of parasites and mammalian cells are known to be blocked by albendazole (ABZ), which ultimately causes cell death by inhibiting glucose absorption and transport. They eventually have parasite-killing effects on ovicidal, larvicidal parasites. Mebendazole and albendazole are widely recommended to treat intestinal nematode infections, such as ascariasis, as well as strongyloidiasis, and hookworm infections. But ABZ also has significant anti-therapeutic effects against tissue nematode infections (visceral, ocular, neural, anisakiasis, trichinosis, angiostrongyliasis, gnathostomiasis, dracunculiasis, cerebral and subcutaneous cysticercosis, and echinococcosis). Lymphatic filariasis, onchocerciasis and dirofilariasis are among the filarial illnesses that are treated with ABZ, either alone or in conjunction with other medications like ivermectin or diethylcarbamazine. Even trematode (fascioliasis and intestinal fluke infections) and protozoan diseases were treated with albendazole (giardiasis, vaginal trichomoniasis, cryptosporidiosis).

3.2.1 Mechanism of action

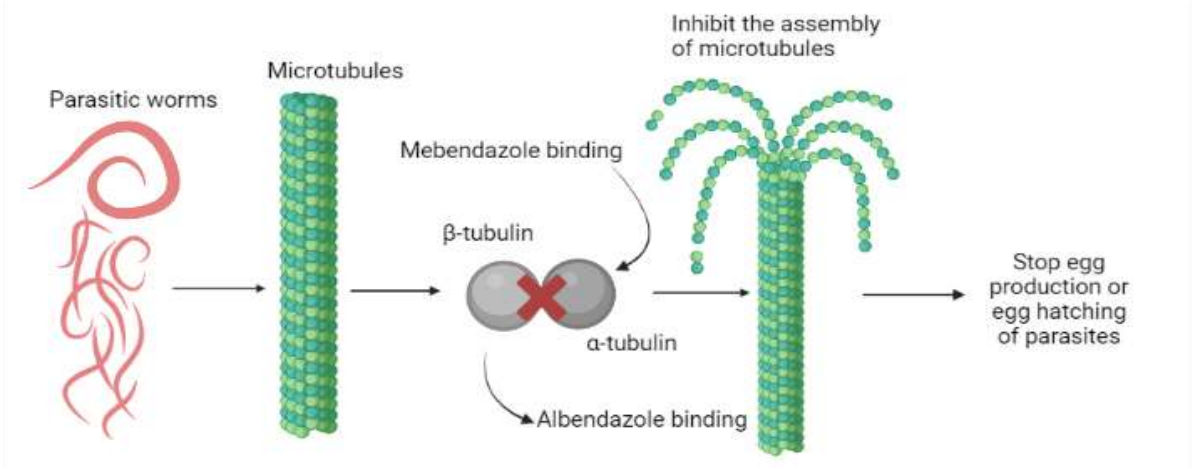

Figure 1: Antiparasitic MoA of ABZ and MBZ

Albendazole and Mebendazole both the drugs have similar mechanism of action. The microtubule of the helminthic cell is formed by the assembly of two globular proteins, alpha and beta tubulin. Mebendazole and Albendazole the anthelminthic drugs inhibit the assembly of both alpha and beta tubulin dimers therefore halts the formation of new helminthic microtubules *(Figure 1).* This function inhibits the growth and development of helminthic cells and leads to cell death in parasites *(Figure 1).*

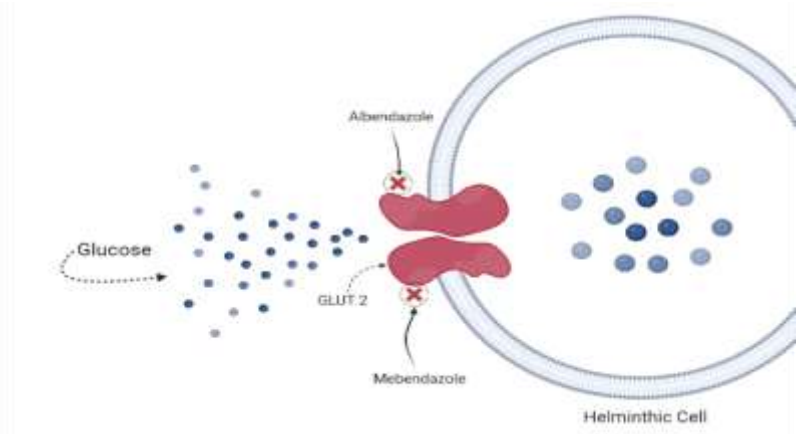

Figure 2: Additional antiparasitic MoA of ABZ and MBZ

Additionally, through different complex mechanisms both ABZ and MBZ inhibit the uptake of essential glucose in helminthic cells *(Figure 2).* Glucose is essential for the locomotion and survival of the parasites. So, with the lack of glucose uptake the parasite gets immobilized and dies.

3.3 Pharmacokinetics of Albendazole and Mebendazole

Effective anthelmintic Albendazole (ABZ) and Mebendazole (MBZ) has been widely used for many years. However, there is a lack of thorough pharmacokinetic (PK) characterization in diverse populations. Numerous clinical and pre-clinical trials have indicated that ABZ and MBZ both can be safe and efficient CRC therapeutic option in Stage IV metastasis and Stage I as well (Son et al., 2020a). But these drugs couldn't operate at their full potential because of their limited bioavailability. For instance, ABZ and its combinations had a very low average bioavailability of about 5–10% during a clinical study that a Swiss Tropical and Public Health center performed in a remote region of the Ivory Coast. Other studies suggests that ABZ has a bioavailability of <5% and MBZ has 5-10% (Hegazy et al., 2022). ABZ also assists in its own absorption. A formulation with high bioavailability attributes for ABZ and MBZ has been under development, with the completion of that formulation these drugs might be used at their full potential (Howard, 2007).

Table 1: Pharmacokinetics of Albendazole

Pharmacokinetics	**Albendazole**			**Reference**
Absorption	**Data types**	**Values**		(Schulz et al., 2019)
	Bioavailability	<5%		
	C_{max}	12.5-26.5 ng/mL		
	T_{max}	2-5 hr		
	AUC	44-78 ng.h/ml		
	Impact of food	Fatty meal speeds absorption		
Distribution	Vd	10mg/kg		(Bloom & Ryan, 2012)
	Plasma protein binding	70%		
	Blood brain barrier (BBB) crossing	Yes		
Metabolism	-	CYP2J2 and CYP2C19		(Wu et al., 2013)
Excretion	Clearance	<1%		(Schulz et al., 2019)
	Half-life($t^{1/2}$)	~1.5 hr		
	Excretion of the drug	Urine	<1%	
		Bile	~98%	

Albendazole's PK characteristics included a median half-life of 1.5 hours, a duration to reach maximum concentration of 2 hours, a maximum concentration C_{max} of 12.5 to 26.5 ng/ml, and an area under the concentration-time curve (AUC) ranging from 44 to 78 ng/h/ml. Fatty meal facilitate the absorption of ABZ. ABZ can cross the blood brain barrier and can exert its impact on CNS. Urinary excretion (<1%) of ABZ is significantly lower than the biliary excretion (~98%). ABZ is metabolized by the two sub enzymes of CYP450 class, CYP2J2 and CYP2C19.

Table 2: Pharmacokinetics of Mebendazole

Pharmacokinetic parameters	**Mebendazole (Approved in US in 1974)**			**Reference**
	Dosage – 10mg/kg			
Absorption	**Data types**	**Values**		(Guerini et al., 2019a)
	Bioavailability	5-10%		
	C_{max}	17.5-500 ng/mL		
	T_{max}	1.5 hr (.5-3hr range)		
	AUC	207.2 ng.h/ml		
	Impact of food	Fatty meal speeds absorption		
Distribution	Vd	1-2L/kg		(Bethesda, 2017)
	Plasma protein binding	90-95%		
	Blood brain barrier (BBB) crossing	Yes		
Metabolism	-	CYP2J2 and CYP2C19		(Hong, 2018)
Excretion	Clearance	<1%		(Pantziarka et al., 2014)
	Half-life($t^{1/2}$)	3-6 hr		
	Excretion of the drug	Urine	<2%	
		Bile	-	
		Feces	~90%	

Mebendazole in its 10mg/kg dose shows 5 to 10% bioavailability on a normal patient regardless of their physiological and pathological conditions. During liver or kidney impairment the values may differ. MBZ shows high protein binding capacity thus might require a higher dose to

formulate. Regarding metabolism MBZ is also metabolized by the same set of enzymes that are responsible for the ABZ metabolism. The excretion rate is significantly higher in feces (~90%) rather than in urine (<2%) and biliary excretion is near zero. The meal's content had a noticeable impact on how well albendazole was absorbed. High fat meals boost the absorption of ABZ and MBZ (Son et al., 2020a). When compared to a low-fat meal, a high-fat breakfast doubled the area under the concentration-time curve (AUC) and maximum concentration (C_{max}) of albendazole, albendazole sulfoxide and MBZ (Ochoa et al., 2021).

Chapter 4

Use of Albendazole and Mebendazole in Colon Cancer Treatment

4.1 Albendazole mode of action on cancer cells*:*

Like paclitaxel interacts with microtubules, ABZ also does so. A-tubulin, which polymerizes to create microtubules, is a key component of the tubulin dimer. Acetyl-a-tubulin, a marker for stable microtubules, was studied to help visualize the microtubule structure (Al-Bassam & Corbett, n.d.). This research demonstrated that paclitaxel, ABZ, and both of their combinations caused the compacted nuclei typical of apoptosis. In contrast, paclitaxel intensified the polymerization of acetylated-a-tubulin as well as a-tubulin. This shows that paclitaxel increases polymerization and stabilizes microtubules, while ABZ promotes depolymerization of microtubules.

4.1.1 Regulation of apoptosis and autophagy in cancer cell

In colorectal cancer cell lines, ABZ can drastically decrease carcinogenesis by up regulating both apoptosis and autophagy. The anti-tumor properties of ABZ were examined in recent research using the human colon adenocarcinoma cell lines HCT-15, HCT-116, HT-29, and SW480 (Jung et al., 2022). Normal cells and colon cancer cell lines were used to examine the cytotoxicity of ABZ. Researchers discovered that ABZ enhanced late apoptotic cells, promoted apoptosis, and caused the subG1 arrest during cell cycle progression (Jung et al., 2022). In CRC cell linings, the broad-spectrum antioxidant N-acetyl cysteine (NAC) may successfully suppress both apoptosis and autophagy (Jung et al., 2022). However, ABZ was able to reverse the effects of NAC-induced reduction of apoptosis and autophagy in colon cancer cells.

4.1.2 Selective apoptotic cell death

Albendazole was found to selectively kill cancer cells, being most potent in the colorectal cancer cell line HT-29, with both drugs having IC_{50} values of less than 1 µM at 48 h. Both mebendazole and albendazole induced classical apoptosis characterized by caspase-3 activation, phosphatidylserine exposure, DNA fragmentation, mitochondrial membrane permeability, and reactive oxygen species production. Cell cycle arrest in the G2/M phase was found, and tubulin polymerization was disrupted (Petersen & Baird, 2021b).

4.1.3 Cell cycle arrest in cancer cells

To ascertain the mechanism behind the decline in cell viability after a 24 or 48-hour treatment with ABZ, research on HT29 CRC cells was conducted. Propidium iodide (PI) fixation and staining were used to examine the progression through the cell cycle. As a positive control, paclitaxel was utilized, which is known to stop the cell cycle in G2/M by attaching to microtubules. The G2/M phase increased considerably after treatment with ABZ or paclitaxel. At 24 hours, paclitaxel was at 62.42 percent and ABZ was at 58.68 percent (Petersen & Baird, 2021). There were concurrent reductions in the percentage of cells in the G1 and S phases and smaller, but still substantial, increases of cells in the G2/M phase (Petersen & Baird, 2021).

4.1.4 ABZ induce DNA fragmentation of cancer cell DNA:

Through the fragmentation of DNA, which is demonstrated by the presence of a unique fragment known as sub-G1 DNA, ABZ can cause apoptosis in CRC cells. A recent study found that, the

percentage of cells with sub-G1 DNA content was considerably raised after both 24 and 48 h of treatments with ABZ. After 24 hours, 11.53 percent (P=0.026) of the treated cells had sub-G1 DNA content (Petersen & Baird, 2021). Antitumor medication interactions with DNA in cancer cells cause cell damage and target cell death (Bischoff et al., 2005). Oxidative damage to CT-DNA caused by ABZ treatment has been investigated favorably since it could provide therapeutic prospects for the treatment of cancer (Hosoya & Miyagawa, 2014). UV light, reactive oxygen and nitrogen species, extrinsic chemical compounds, and others may all change the structure of DNA (Mikhed et al., 2015). Products of DNA degradation are produced by drugs that may oxidize DNA. Because ABZ therapy produces too much ROS, it can oxidize DNA, which damages cancer cells' DNA.

4.2 Mebendazole mode of action on cancer cell

MBZ blocks a broad variety of tumor-promoting processes, including tubulin polymerization, angiogenesis etc. In addition to its direct cytotoxic effects, mebendazole also interacts favorably with ionizing radiation, several chemotherapeutic drugs, and the immune system's antitumor response.

4.2.1 Inhibition of tubulin polymerization

In cancer cells, mebendazole binds to tubulin dimers to stop them from forming microtubules. MBZ pushes the cancer cell into mitotic cell attest, initiating tubulin depolymerization, in contrast to other existing microtubule inhibitors. The antiproliferative action of MBZ has been shown by a variety of in vivo and in vitro experiments, and it is now undergoing trials. The antiproliferative and tubulin depolymerization effects of MBZ were evaluated in vivo using a mouse model(Guerini

et al., 2019b). discovered a significant tumor growth inhibition. The microtubule structure is disturbed by MBZ, which also prevents cancer cells from migrating and invading. When treating CRC and GIT cancer cell lines like Texans, MBZ has shown stronger efficacy in terms of antiproliferative effect than other currently available microtubule inhibitors, notably paclitaxel(Guerini et al., 2019a). Additionally, when combined with another chemotherapy agent like 5-FU, MBZ has stronger anticancer effects than when used alone (Guerini et al., 2019).

4.2.2 Inhibition of angiogenesis in progressive tumor

The growth of a blood supply to an already-existing tumor, or angiogenesis, accelerates colorectal cancer spread. CRC begins with the development of polyps in the rectum and colon, which grow into malignant entities with the potential to spread by metastasis. Angiogenesis plays a significant role in cancer cell growth and metastasis since a significant portion of CRC is discovered in advanced stages. Inhibiting angiogenesis in tumors may prevent the spread and growth of already-existing tumors and may also be a key factor in the development of CRC. It has been shown that MBZ is a crucial therapeutic technique for preventing angiogenesis in CRC tumors. Through in vivo testing using a mouse model and the dorsal air sac technique, the angiogenesis inhibitory action of MBZ was assessed, and it was discovered to be suppressing angiogenesis in a compelling way (Bai et al., 2015). Human umbilical vein endothelial cells (HUVECs) were used to demonstrate the anti-angiogenic impact of MBZ, which revealed symptoms of angiogenesis suppression in a dose-dependent manner (Sung et al., 2019). Additionally, MBZ causes an increase of p53, which aids CRC cells in arresting their cell cycle (Sung et al., 2019).

4.2.3 Antitumor immune response induction

Mebendazole may cause patients' immune systems to respond by fighting CRC metastasis. Numerous investigations and clinical trials have shown this impact. Some in vivo and in vitro investigations point to a mechanism through which MBZ boosts the immune system of patients. Examples include the proinflammatory (M1) and anti-inflammatory (M2) inflammatory mediation systems that the human body's monocyte-macrophage system exhibits (M2) (Guerini et al., 2019a). The M1 macrophages exhibit phagocytic, antigen-presenting, and cytotoxic activities (Blom et al., 2017). T lymphocytes and natural killer (NK) cells are two examples of immune system cells that may be activated by M1 macrophages. According to one research, MBZ has a proinflammatory (M1) tumor-suppressive impact on macrophages and human leukemia monocytic cell line (Choi et al., 2022). When MBZ was used, pro-inflammatory (M1) genes that code for cytokines like TNF, IL8 and IL6, surface markers like CD80 and CD86, and chemokines that draw in T cells were significantly upregulated (Blom et al., 2017). In a co-culture setup with differentiated THP-1 macrophages and HT29 colon cancer cells, MBZ clearly triggered a tumor suppressive effect. Additionally, MBZ showed strong inhibitory effect against DYRK1B, also known as dual specificity tyrosine phosphorylation, regulated kinase 1B (Blom et al., 2019). The immune system may be modulated by the enzyme DYRK1B. The inquiry into MBZ's behavior is now ongoing. The DYRK1B inhibitory impact and proinflammatory(M1) polarization, especially in macrophages, suggest that inhibition of this DYRK1B kinase may partially recapitulate immunological responses caused by MBZ, as previously demonstrated, in human leukemia monocytic cell line and macrophages (Blom et al., 2019).

4.3 Clinical evidence of anti CRC activity of ABZ and MBZ

Albendazole (ABZ) has already been used as an antiparasitic drug in wide range of anthelminthics treatment and proved to be efficacious and safe in wide range of population (Chai et al., 2021). But its anticancer properties are yet to be fully uncovered. Some clinical and preclinical trails proved its potential to be used as a safe and effective choice of medication, this drug still needs a lot of field tests and clinical trials to be reached to its full potential. ABZ has already been proved to be effective in clinical trials regarding CRC, liver cancer of hepatocellular carcinoma, GI tract cancer, pancreatic cancer, lung cancer, ovarian cancer, prostate, and biliary cancer as well as for untreatable malignant forms of cancer and this study is still ongoing with 250 participants with fatal and untreatable malignant form of cancer (Chai et al., 2021). Following two courses of treatment with capecitabine, oxaliplatin, and bevacizumab and subsequently capecitabine and irinotecan, a 74-year-old patient with metastatic colon cancer in progression in various locations (lungs, abdominal lymph nodes, and liver) was documented in a clinical trial report (Nygren & Larsson, 2014). After six weeks of monotherapy with Mebendazole at a dosage of 100 mg twice daily, a CT scan revealed almost full remission of the metastases in the lungs and lymph nodes as well as a good partial remission in the liver (Nygren & Larsson, 2014). The patient didn't have any negative side effects, but the medication had to be briefly discontinued since the liver enzymes had momentarily increased. Six active and recruiting clinical trials has been going on to observe the therapeutic effectiveness of Mebendazole (MBZ) three of them specifically focused on MBZ in CRC treatment. ABZ has been under clinical trial in recruiting stage for CRC in three different ongoing trial and one trial for refectory solid tumor. All these clinical trial data are available in clinicaltrails.gov website and summarized in Table 3 and Table 4.

Table 3: Summarization of albendazole clinical use for CRC

Drug	Disease condition	Number of Patients	Drug regimen	Side effects	Summary of findings	References
Albendazole	CRC and HCC	7 patients	10 mg/day	Neutropenia in patients 1,2 and 8	ABZ use showed an extensive tumor biomarker stabilization	(Chai et al., 2021)
	Refectory solid tumor	36 patients	400mg-1200mg/day (Oral)	Fatigue, mild GI upset	1 patient, stable marker response, 16%, tumor marker responses with drops of 50% or more.	(Morris et al., 2001)
	CRC	12 patients	800mg/day	No severe side effects	Maximum acceptable dose of ABZ determined (Phase I)	(Pourgholami et al., 2010)
	Malignant Disease, CRC	250 patients	Not mentioned	No severe side effects	Phase 2 clinical trial (Albendazole)	(Son et al., 2020b)

Table 4: Summarization of albendazole clinical use for CRC:

Drug	Disease condition	Number of Patients	Drug regimen	Side effects	Summary of findings	References
Mebendazole	CRC	1	200mg/day for 42 days	No severe side effects	Near complete remission	(Nygren & Larsson, 2014)
	CRC (Stage 4)	40	Given as an adjuvant therapy	No severe side effects	Phase 3 clinical trail	(Hegazy et al., 2022)
	Variable cancers	207	100mg/day	No severe side effects	Phase 3 clinical trail	(Son et al., 2020a)
	Malignant Disease, CRC	250 patients	Not mentioned	No severe side effects	Phase 2 clinical trial (Mebendazole)	(Son et al., 2020a)

A study was being done on albendazole (ABZ) to explore whether it might be used to treat CRC and HCC. The experiment began with 7 patients receiving 10 mg of albendazole daily, three of whom had neutropenia as a side effect. However, the medicine demonstrated its promise by stabilizing the widespread tumor markers in these individuals. Like this, 36 patients were participating in an albendazole study for resistant solid tumors. The patients received 400–1200 mg of ABZ daily. We saw a few typical side effects, such as weariness and a slight GI discomfort. The trial's findings showed that four (16 percent) of the 24 patients who could be evaluated had tumor marker responses with declines of at least 50% from baseline levels, and one patient had a

lengthy duration of sustained marker response. once again, ABZ was being tested on 12 patients for CRC. Phase 1 of the clinical study was used to identify the maximum tolerated dosage. Patients in this experiment used this medication 800 mg/day and had no serious adverse effects. With 250 patients, ABZ is undergoing another phase 2 clinical research to determine its impact on malignant illness. Mebendazole (MBZ) is undergoing a study with only one patient to see if it could cure CRC. The patient used this medication for 42 days at a dose of 200 mg per day with no serious adverse effects. Right present, the patient's remission is almost complete. In a different study, MBZ was administered as adjuvant treatment to 40 patients in stage 3 of the clinical trial for stage 4 CRC, and there were no serious adverse effects. Phase 3 of another MBZ clinical study with 207 individuals is being conducted for the treatment of various malignancies. They are given 100 mg of this medication every day. Finally, MBZ is now undergoing a phase 2 clinical study for malignant illness and CRC, which is being conducted with 250 patients. For various kinds and stages of cancer, clinical studies are being conducted using the benzimidazole medicines ABZ and MBZ. While the clinical trials are currently recruiting participants, some in vivo and in vitro trial data has been published. The outcomes of the clinical trials that have been completed so far in treating CRC for both ABZ and MBZ have been good. To demonstrate the therapeutic efficacy of ABZ and MBZ in the treatment of CRC, further trials must be done.

Chapter 5

5.1 Conclusion and future perspective

Albendazole and Mebendazole are both powerful, broad-spectrum anti-parasitic and anti-cancer medications. The use of ABZ and MBZ has led to the effective management of human intestine helminthiases (nematodes and cestodes), tissue helminthiases (nematodes and cestodes), filarial nematode infections, liver and intestinal trematode infections, and various protozoan disorders. This medicine has lately received attention because to its anti-cancer abilities in vitro and in vivo of animals, as well as a few reported clinical anti-cancer studies. Before being taken into consideration as a treatment option for CRC, they should go through further testing. ABZ and MBZ are highly safe anti-parasitic medications when used as prescribed. Care must be exercised, nevertheless, since liver damage may rarely occur in individuals who get only one dose of ABZ and MBZ. More and more instances of parasites developing ABZ resistance have been reported. Both medications are secure and well-known on the market, but it is yet unknown how effectively they will work to treat CRC at both the early and later stages. The outcomes of various current studies concentrating on the use of ABZ in CRCs in the early stages and MBZ in CRCs in the later stages may reveal the full potential of these drugs. Both medications have a very poor bioavailability; thus, many factors need to be taken into consideration. Research must be done to develop novel ways to deal with this problem.

5.2 Limitations

Solubility is known to be a need for medication absorption, bioavailability, and therapeutic response. Since ABZ applications' solubility is a limiting constraint, formulating into nanostructures may be a great option for improving ABZ solubility, bioavailability, and delivery effectiveness. There are now six different kinds of ABZ nano formulations produced for these uses in different nanostructures (Movahedi et al., 2017). The solubility of ABZ has been increased by these nanostructured nano formulations, which also include host-guest nanocomplexes, liposomes, different polymeric, and SL nanoparticles. Ehrlich ascites carcinoma-bearing mice were employed in an in vivo experiment to confirm the anticancer activity (Castro et al., 2016). The results revealed that when using ABZ (20 mg/kg) and MTX (2 mg/kg) as treatment alternatives, tumor development was inhibited by 32%, while MTX exhibited a 55% decrease in tumor growth (Castro et al., 2016). Treatments with MTX and ABZ both increased the animals' survival times. Because of the potential relationship between the limited bioavailability of ABZ and the differences in its effects, pharmacokinetic and structural activity investigations are required to increase the drug's absorption hench ramping up the bioavailability.

5.3 Potential solutions

The usage of nano formulations of ABZ as both an anticancer and an antiparasitic agent may improve this scenario. This drug's limited oral bioavailability has been shown to be one of the main obstacles for researchers in repositioning it as an effective anticancer medicine. Using NP formulation of ABZ has been proved to be advantageous through several studies done by several forms of ABZ NP formulation like, albumin, chitosan, PLGA nanoparticle. Several of those studies

showed better bioavailability, better therapeutic impact with lower dose and better absorption. Some of the study results are provided in *(Table 5).*

Table 5: ABZ and its NP formulations

Type of nano-formulation	**Dosage**	**Application**	**Results**	**Advantage**	**References**
M1. albendazole	0.5-3.2mg/kg	On mice (Preclinical)	Mice's survival rate improved.	Low dose	(Hettiarachchi et al., 2016)
Albumin nanoparticle	0.09-1.88uM	On mice (Preclinical)	Reduced tumor development in mice	Less harmful than free ABZ	(Noorani et al., 2014)
Chitosan nanoparticle	100ug/ml	Human liver cancer cell	Reduction in the viability of cancer cells	Biocompatible and sustainable formulation	(Ehteda et al., 2012)
PLGA nanoparticles	100ug/ul	Human ovarian cancer cell	More DNA fragmentation was observed compared to free ABZ	Stability and stronger adherence at the region of absorption	(Kang et al., 2017)

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