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An investigation of 5-Fluorouracil in colorectal cancer: Pharmacological efficacy, invitro and invivo studies

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ABSTRACT :

Colorectal cancer (CRC) is among the leading causes of cancer-related mortality worldwide. This study evaluates the role of 5-fluorouracil (5-FU) in CRC treatment by reviewing its mechanism, pharmacology, and experimental models. The report critically examines its monotherapy efficacy and its performance in combination regimens like FOLFOX and FOLFIRI. In vitro studies on HT-29 cells and in vivo experiments in murine models were assessed for pharmacodynamics, toxicity, and stability. Further, the study discusses formulation innovations and therapeutic strategies aimed at improving 5-FU efficacy. This comprehensive analysis underlines the enduring significance of 5-FU in CRC management and highlights future prospects such as personalized medicine and advanced drug delivery systems.

Keywords: Colorectal cancer, 5-Fluorouracil, In vitro, In vivo, Chemotherapy, FOLFOX, Pharmacodynamics

INTRODUCTION

A collection of aberrant cells that develop out of control by disregarding the standard norms of cell division are referred to as cancer. Signals that determine whether a normal cell should divide, develop into a different type of cell, or die are continuously sent to the cell. These signals provide cancer cells some autonomy, which leads to unchecked growth and multiplication.

COLORECTAL CANCER

The distinct type of cancer that affects the colon or rectum is recognized as colorectal cancer (CRC). It is amongst the most prevalent forms of cancer in the globe. After prostate, breast, and lung cancers, cancer ranks the fourth in terms of incidence. Depending on the initial site of genesis, these tumors are known as either rectal or colon cancer.[1] In its early stages, colorectal cancer frequently exhibits no symptoms.

Typical signs and symptoms include: alterations in bowel habits, such as constipation, diarrhea, or constipation, stool with blood, cramping in the abdomen, bizarre loss of weight, fatigue and Anaemia due to iron shortage

1.1 TUMOR TYPES

In accordance with macroscopic appearance:

- The right colon is usually the site of a fungating exophytic lesion.
- The left colon is where flat infiltrative lesions are most frequently observed.

According to microscopic appearance:

- over 85% of colorectal cancers are tubular adenocarcinomas.
- Ten to fifteen percent of cases had mucinous adenocarcinoma, which is linked to a shorter lifespan.[2]

1.2 RISK ELEMENTS

The following variables may raise the chance of acquiring colorectal cancer:

1. Age: People over 50 are at higher risk.
2. Family history: genetic syndromes (e.g., Lynch syndrome, FAP) or colorectal cancer.

3. Personal history: Adenomatous polyps or CRC in the past.
4. Aspects of lifestyle: low in fiber and heavy in processed meats.[3]

ETIOLOGY

The majority of colorectal cancers are sporadic. A two-step carcinogenesis model comprising initiation and promotion is thought to be the mechanism by which colorectal cancer develops.

Initiation: The precise initiators are unknown. Food processing may produce suspected carcinogens.

Promotion: Environmental exposure and genetic predisposition work together to promote carcinogenesis.[4] [5]

1.4 PATHOPHYSIOLOGY

The Lieberkühn crypts are at which the colon's cell turnover takes place. When cells divide, exposure to toxins damages their DNA. Defective DNA repair causes cells to develop into initiator cells. As a result of prolonged exposure, these cells can transform into cancerous cells that generate polyps.

1.5 MEDICATIONS USED FOR COLORECTAL CANCER

Among the frequently utilized medications are:

5-FU, or fluorouracil, Capecitabine, Irinotecan, Oxaliplatin, Tipiracil/trifluridine [6]

2. 5-FLUOROURACIL [5-FU]

One chemotherapeutic agent used to treat an array of neoplasms is 5-fluorouracil (5-FU). It is a pyrimidine antagonist, an antineoplastic agent that is a member of the antimetabolite class.

5-FU is a prodrug that is processed down by the body into a deoxyribonucleotide, which is its active form. Because it prevents pyrimidines from forming normally, this active form disrupts DNA synthesis, which in turn prevents the growth of cancer cells.

Usually, a dose of 12 mg/kg/day is given intravenously for four days in sequence.

2.1 MECHANISM

The body transforms 5-fluorouracil to 5-fluoro-2'-deoxyuridine monophosphate (FdUMP). Thymidylate synthase, the enzyme that converts deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP), a vital step in DNA synthesis, is inhibited by this active metabolite. FdUMP hinders DNA synthesis by blocking thymidylate synthase, which interferes with thymidine synthesis and causes an imbalance in thymidine nucleotides. Furthermore, 5-FU and its metabolites may also be integrated into DNA and RNA, disrupting their regular functions and causing cytotoxicity in cancer cells that divide quickly.

2.2 TYPICAL 5-FLUOROURACIL (5-FU) SIDE EFFECTS

Nausea, tiredness (fatigue), diarrhea, changes in skin pigmentation, mucositis, reduced bone marrow activity.

Less frequent adverse effects include blurred eyesight, appetite loss, rashes on the skin, thinning of the hair, skin conditions, myelosuppression [7]

2.3 5-FLUROURACIL ASSAY

- **Choosing the Right Solvent for Absorbance:** Weighing and testing about 20 mg of 5-fluorouracil in a range of solvents revealed that it was soluble in: distilled water, 0.1 N NaOH, DMF or dimethylformamide, Water of HPLC quality
  After five minutes of sonication, it was discovered that the medication was soluble in HPLC-grade water.
- **Standard Stock Solution Preparation:** A 50 ml volumetric flask was filled with precisely weighed 20 mg of 5-fluorouracil. It was sonicated for five minutes to dissolve it in HPLC-grade water. To get a 400 µg/ml stock solution, the volume was increased to 50 ml using the same solvent. To create a 40 µg/ml solution, 5 ml of the stock solution mentioned above was pipetted and diluted with 50 ml of HPLC-grade water.
- **Making the Working Standard Solution:** To get a final working concentration of 8 µg/ml, the intermediate solution was suitably diluted further.
- **Wavelength (λmax) Selection for Analysis**: 20 µg/ml of 5-fluorouracil was produced as a solution. Using HPLC-grade water as the blank, the UV spectra was recorded between 200 and 400 nm. At 265 nm, the highest absorbance (λmax) was recorded.[8]

3. 5-FLUOROURACIL AN INVITRO AND INVIVO INVESTIGATION IN COLORECTAL CANCER

3.1 INVITRO STUDY

Every animal experiment was carried out in accordance with ethical standards that were authorized by Alexandria University's Faculty of Science's Institutional Animal Care and Use Committees (IACUCs). The animals were kept in optimal temperature, humidity, and light cycles, and they had free access to water and normal pellet diet. An acclimatization period of one week was observed prior to the start of the trials.[9]

- **IC50 determination:**

Principle:

The half-maximal inhibitory concentration, or IC_{50}, is a common indicator of a substance's ability to block a certain biological or metabolic activity. It stands for the concentration needed to stop half of the target activity (e.g., cell proliferation, enzyme). [10]

Procedure:

Six-well plates were used to raise colorectal adenocarcinoma cells, which were then exposed to different 5-fluorouracil concentrations. Trypsin/EDTA was used to extract cells. It is separated after being resuspended in PBS, with one part used for homogenate preparation and the other for RNA isolation. Using a commercial RNA isolation kit, RNA was isolated, and its purity was assessed using spectrophotometry at 260 nm. Homogenate prepared using a homogenizer in phosphate buffer, cells were lysed. The supernatant, which contained proteins, RNA, and other substances, was separated from the lysates by centrifugation and used for additional biochemical tests.[9]

- **MTT Assay:**

Principle:

The MTT assay employs mitochondrial enzymes in living cells to transform yellow tetrazolium MTT to purple formazan, which is the substance that assesses cell viability. At 540–570 nm, absorbance is measured.[11]

procedure:

HT-29 cells were exposed to varying amounts of:

- 5-Fluorouracil
- PEG-CS MPs coated with blank CAP
- 5-FU-loaded PEG-CS MPs covered with CAP

MTT solution was added and incubated for three hours following 48–72 hours of incubation at 37°C in 5% CO_2. After removing the supernatant, propanol was added to dissolve the formazan. At 540 nm, absorbance was measured.[12]

- **Stability Study**

Principle:

Stability studies assess the ability of a drug to retain its biological, chemical, and physical integrity under an array of storage conditions.[13]

procedure:

For 79 days, PVC infusion bags containing 800 mg/100 mL of 5-FU were frozen at -20°C.After being thawed in the microwave, the bags were kept for 28 days at 5°C ± 3°C.

Measurement of HPLC concentration, Visual and microscopic inspections as well as pH monitoring were carried out.

If ≥90% of the initial drug concentration was maintained with 95% confidence, the solution was deemed stable.[14]

- **Flow Cytometry**

Principle:

Using light scattering, laser excitation, and fluorescent markers, flow cytometry evaluates cellular properties. It permits in-depth investigation of the cell cycle.[15]

procedure:

Cells were subjected to either a 1-hour or 72-hour exposure to cytotoxic doses of 5-FU. A 72-hour exposure caused S-phase arrest, just like 5-fluoro-2'-deoxyuridine. S-phase accumulation was transient after 1 hour of exposure, similar to 5-fluorouridine. This demonstrated that the manner in which 5-FU acts changes with exposure time frame.[16]

- **COMET assay**

Principle:

sometimes referred to as single-cell gel electrophoresis, detects breaks in DNA strands by looking for "comet tails" that emerge as damaged DNA migrates during the electrophoresis process.[17]

procedure:

The cells were placed onto slides after being suspended in PBS and combined with low-melting-point agarose. After being lysed at 4°C and exposed to alkaline buffer, the slides were electrophoresed. SYBR Green or ethidium bromide were used to stain the DNA, and fluorescence microscopy was used

to view the results. DNA damage was evaluated by quantifying the length and intensity of the tail.[18]

3.2 INVIVO STUDY

The pharmacodynamic and toxicological profiles of medications in living creatures are assessed in in vivo investigations, which offer practical insights into ADME characteristics and treatment effectiveness.[19]

Method:

Standard laboratory settings were used to maintain 105 inbred albino mice that were randomly assigned. 1,2-dimethylhydrazine (DMH), 20 mg/kg BW, was administered intraperitoneally once a week for eight weeks in order to develop colorectal cancer.

Prophylactic group:

GROUP	NUMBER OF ANIMALS	DURATION	TREATMENT
Group 1 Negative control)	7 mice	16 weeks	Normal
Group 2 (Sham control)	7 mice	16 weeks	Saline solution
Group 3 (Positive control)	10 mice	16 weeks	• Saline solution (8 weeks) • 1,2-Dimethylhydrazine IP injection (8 weeks)
Group 4 (Sericin prophylactic group)	7 mice	16 weeks	• 75mg sericin/kg (8 weeks) • Carcinogenic agent (8 weeks)
Group 5 (Propolis prophylactic group)	7 mice	16 weeks	• 75mg propolis/kg (8 weeks) • Carcinogenic agent (8 weeks)
Group 6 (Nanoparticle sericin/ propolis prophylactic group)	7 mice	16 weeks	• 75 mg sericin/propolis nanoparticles/Kg • Carcinogenic agent (8 weeks)

Table 1: prophylactic treatment group

In order to verify the presence of the tumor using colon histology, three mice from Group 3 were subjected to death prior to the conclusion of the experiment.

Treatment group:

GROUP	NUMBER OF ANIMALS	DURATION	TREATMENT
Group 1 (Negative control)	7 mice	16 weeks	Normal
Group 2 (Sham control)	7 mice	16 weeks	Saline solution
Group 3 (Positive control)	10 mice	16 weeks	• 1,2-Dimethylhydrazine IP injection (8 weeks) • Saline solution (8 weeks)
Group 4I	7 mice	16 weeks	• Carcinogenic agent • 75 mg sericin/Kg
Group 4II	7 mice	16 weeks	• Carcinogenic agent • 75mg propolis/kg
Group 4III	7 mice	16 weeks	• Carcinogenic agent • 75 mg sericin/propolis nanoparticles/Kg

Group 4IV	7 mice	16 weeks	• Carcinogenic agent • sericin/propolis/5-Fluorouracil/Kg
Group 4V	7 mice	16 weeks	• Carcinogenic agent • 75 mg 5-Fluorouracil/Kg

Table 2: cancer treatment group

All animals were subjected to death by isoflurane overdose at the conclusion of the 16-week period, and colon specimens were collected for: Analysis of histology, assays that use biochemistry, molecular characterization.[9]

5-FLUOROURACIL MONOTHERAPIES

5-Fluorouracil as a Monotherapy in Colorectal Cancer 5-Fluorouracil (5-FU) is an antimetabolite used in chemotherapy and used to be the mainstay of chemotherapy for colorectal cancer (CRC).

Adjuvant Treatment

5-FU dramatically lowers the chance of recurrence following curative surgery for Stage II and III colon cancer.

palliative treatment

Patients that are elderly or fragile. Individuals with poor performance status or organ malfunction and situations in which combination therapy is not practical.

Sequential Use and Maintenance Therapy

5-FU monotherapy could be used as after combination induction treatment.

The two major risks are neurotoxicity and cardiotoxicity. 5-FU toxicity can be reversed with uridine triacetate, a pyrimidine analog, if administered within 96 hours. By competing with 5-FU metabolites, it suppresses their incorporation into RNA/DNA.[20]

4.1 FORMULATIONS

(IV) Bolus: Given over a period of two to four minutes. raises peak levels but is linked to increased hematologic toxicity.

Continuous Infusion: given for 24 to 96 hours at a time. provides longer exposure with improved efficacy with fewer adverse effects.

Oral Prodrugs

Oral prodrugs are employed because 5-FU is poorly absorbed orally. Capecitabine and Tegafur are two examples.[21]

COMBINATION THERAPY IN COLORECTAL CANCER

Regimen	Components
FOLFOX	5-fluorouracil + Leucovorin + Oxaliplatin
FOLFIRI	5-fluorouracil+ Leucovorin + Irinotecan
FOLFOXIRI	5-fluorouracil+ Leucovorin + Irinotecan + Oxaliplatin
CapeOX	Capecitabine + Oxaliplatin

Table 3: combination therapies for colorectal cancer [22]

6. FUTURE PROSPECTIVES

5-FU is still evolving despite its age. Future paths consist of:

- Pharmacogenomics and Personalized Medicine
- Utilizing DPYD gene testing to customize dosage
- Decrease toxicity and boost effectiveness
- Innovative Drug Delivery Methods
- Liposomes, implants, and nanoparticles for targeted delivery
- Combined with New Therapeutic Approaches
- Combining targeted agents, radiosensitizers, or immunotherapies
- Biomarker-Assisted Surveillance
- Formulations & Oral Prodrugs
- Creation of advanced oral forms to improve patient adherence

- Overcoming Opposition
- Finding and using adjunct drugs to address resistance pathways [20]

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