CHAPTER - 2

DRUG AND EXCIPIENT PROFILE

2.1 Methotrexate
2.2 Doxorubicin HCl
2.3 5-Fluorouracil
2.4 Pectin
2.5 Casein
2.1 METHOTREXATE

- Molecular formula of methotrexate is $C_{20}H_{22}N_8O_5$
- Chemical IUPAC Name is (2S)-2-[[4-[(2,4-diaminopteridin-6-yl)methyl-methyl-amino] benzoyl] amino] pentanedioicacid
- Molecular weight is 454.44 g/mol
- Methotrexate available: 10mg tablets

Methotrexate, MTX, known as amethopterin, is an antimetabolite and antifolate drug. It is used in treatment of cancer, autoimmune diseases, ectopic pregnancy, and for the induction of medical abortions. It acts by inhibiting the metabolism of folic acid. Methotrexate began to replace the more toxic antifolate aminopterin starting in the 1950s. The drug was developed by Yellapragada Subbarao.

2.1.1 Mechanism of Action

Methotrexate competitively inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis. The affinity of methotrexate for DHFR is about one thousand-fold that of folate. DHFR catalyses the conversion of dihydrofolate to the active tetrahydrofolate. Folic acid is needed for the de novo synthesis of the nucleoside thymidine, required for DNA synthesis. Also, folate is needed for purine base
synthesis, so all purine synthesis will be inhibited. Methotrexate, therefore, inhibits the synthesis of DNA, RNA, thymidylates, and proteins.

Methotrexate acts specifically during DNA and RNA synthesis, and thus it is cytotoxic during the S-phase of the cell cycle. Logically, it therefore has a greater toxic effect on rapidly dividing cells (such as malignant and myeloid cells, and gastrointestinal and oral mucosa), which replicate their DNA more frequently and thus inhibits the growth and proliferation of these noncancerous cells, causing other side effects also. Facing a scarcity of dTMP, rapidly dividing cancerous cells undergo cell death via thymineless death.

For the treatment of rheumatoid arthritis, patients should supplement their diets with folate. In these cases, inhibition of DHFR is not thought to be the main mechanism, but rather the inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine, or the inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells.

2.1.2 Pharmacokinetics

Methotrexate is a weak dicarboxylic acid with pKa 4.8 and 5.5, and thus it is mostly ionized at physiologic pH. Oral absorption is saturatable and thus dose-dependent, with doses less than 40 mg/m² having 42% bioavailability and doses greater than 40 mg/m² only 18%. Mean oral bioavailability is 33% (13-76% range), and there is no clear benefit to subdividing an oral dose. Mean intramuscular bioavailability is 76%.

Methotrexate is metabolized by intestinal bacteria to the inactive metabolite 4-amino-4-deoxy-N-methylpteroyl acid (DAMPA), which accounts for less than 5% loss of the oral dose.
Factors that decrease absorption include food, oral nonabsorbable antibiotics (e.g. vancomycin, neomycin, and bacitracin), and more rapid transit through the gastrointestinal tract (GI) tract, such as diarrhea, while slower transit time in the GI tract from constipation will increase absorption. Methotrexate is also administered in the placenta accreta, inhibiting the blood circulation to the target site.

### 2.1.3 Side Effects

Acne; chills and fever; dizziness; flushing; general body discomfort; hair loss; headache; infertility; irregular periods; itching; loss of appetite; lowered resistance to infection; miscarriage; nausea; sensitivity to sunlight; sore throat; speech impairment; stomach pain; swelling of the breast; unusual tiredness; vaginal discharge; vomiting.

Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); black or bloody stools; blood in the urine; bone pain; calf pain/swelling; change in amount of urine; chest pain; confusion; dark urine; diarrhea; dry cough; enlarged glands; fatigue; fever or chills; inflammation of the pancreas (stomach tenderness, nausea, vomiting, fever, increased pulse rate); irregular heartbeat; mental changes; mouth sores; muscle weakness; persistent sore throat; red, swollen, or blistered skin; seizures; serious infection (herpes, hepatitis, blood infection); trouble breathing; unusual bleeding or bruising; unusual pain and discoloration of the skin; vision changes; vomit that looks like coffee grounds; yellowing of skin or eyes.

### 2.1.4 Interactions

Concomitant administration of some NSAIDs with high dose methotrexate therapy has been reported to elevate and prolong serum methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity.
Caution should be used when NSAIDs and salicylates are administered concomitantly with lower doses of methotrexate. These drugs have been reported to reduce the tubular secretion of methotrexate in an animal model and may enhance its toxicity.

Despite the potential interactions, studies of methotrexate in patients with rheumatoid arthritis have usually included concurrent use of constant dosage regimens of NSAIDs, without apparent problems. It should be appreciated, however, that the doses used in rheumatoid arthritis (7.5 to 15 mg/week) are somewhat lower than those used in psoriasis and that larger doses could lead to unexpected toxicity.

Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored.

Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria.

Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

The potential for increased hepatotoxicity when methotrexate is administered with other hepatotoxic agents has not been evaluated. However, hepatotoxicity has been reported in such cases. Therefore, patients receiving concomitant therapy with methotrexate and
other potential hepatotoxins (e.g., azathioprine, retinoids, sulfasalazine) should be closely monitored for possible increased risk of hepatotoxicity.

Methotrexate may decrease the clearance of theophylline; theophylline levels should be monitored when used concurrently with methotrexate.

Certain side effects such as mouth sores may be reduced by folate supplementation with methotrexate.

Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression in patients receiving methotrexate, probably by an additive antifolate effect.

### 2.1.5 Contraindications

Methotrexate can cause fetal death or teratogenic effects when administered to a pregnant woman. Methotrexate is contraindicated in pregnant women with psoriasis or rheumatoid arthritis and should be used in the treatment of neoplastic diseases only when the potential benefit outweighs the risk to the fetus. Women of childbearing potential should not be started on methotrexate until pregnancy is excluded and should be fully counseled on the serious risk to the fetus should they be come pregnant while undergoing treatment. Pregnancy should be avoided if either partner is receiving methotrexate; during and for a minimum of three months after therapy for male patients, and during and for at least one ovulatory cycle after therapy for female patients. (Because of the potential for serious adverse reactions from methotrexate in breast fed infants, it is contraindicated in nursing mothers.

Patients with psoriasis or rheumatoid arthritis with alcoholism, alcoholic liver disease or other chronic liver disease should not receive methotrexate.
Patients with psoriasis or rheumatoid arthritis who have overt or laboratory evidence of immunodeficiency syndromes should not receive methotrexate.

Patients with psoriasis or rheumatoid arthritis who have preexisting blood dyscrasias, such as bone marrow hypoplasia, leukopenia, thrombocytopenia or significant anemia, should not receive methotrexate.

Patients with a known hypersensitivity to methotrexate should not receive the drug.

2.1.6 Overdoses

Leucovorin is indicated to diminish the toxicity and counteract the effect of inadvertently administered overdosages of methotrexate. Leucovorin administration should begin as promptly as possible. As the time interval between methotrexate administration and leucovorin initiation increases, the effectiveness of leucovorin in counteracting toxicity.

In cases of massive overdosage, hydration and urinary alkalinization may be necessary to prevent the precipitation of methotrexate and/or its metabolites in the renal tubules.

Generally speaking, neither hemodialysis nor peritoneal dialysis has been shown to improve methotrexate elimination. However, effective clearance of methotrexate has been reported with acute, intermittent hemodialysis using a high-flux dialyzer.

In postmarketing experience, overdose with methotrexate has generally occurred with oral and intrathecal administration, although intravenous and intramuscular overdose have also been reported.

Reports of oral overdose often indicate accidental daily administration instead of weekly (single or divided doses). Symptoms commonly reported following oral overdose include those symptoms and signs reported at pharmacologic doses, partially hematologic and gastrointestinal reaction. For example, leukopenia, thrombocytopenia, anemia, pancytopenia, bone marrow suppression, mucositis, stomatitis, oral ulceration, nausea,
vomiting, gastrointestinal ulceration, gastrointestinal bleeding. In some cases, no
symptoms were reported. There have been reports of death following overdose. In these
cases, events such as sepsis or septic shock, renal failure, and aplastic anemia were also
reported.

2.1.7 Clinical Use

Chemotherapy

Methotrexate was originally developed and continues to be used for chemotherapy either
alone or in combination with other agents. It is effective for the treatment of a number of
cancers including: breast, head and neck, leukemia, lymphoma, lung, osteosarcoma,
bladder, and trophoblastic neoplasms.

Pregnancy termination

Methotrexate is commonly used (generally in combination with misoprostol) to
terminate pregnancies during the early stages (i.e., as an abortifacient). It is also used to
treat ectopic pregnancies.

Autoimmune disorders

It is used as a treatment for some autoimmune diseases including: psoriasis and psoriatic
arthritis, Crohn's disease, and rheumatoid arthritis. It has also been used for multiple
sclerosis but is not approved by the Food and Drug Administration.

Administration

It can be taken orally or administered by injection (intramuscular, intravenous,
subcutaneous, or intrathecal). Oral doses are taken weekly not daily. Routine monitoring
of the complete blood count, liver function tests, and creatine are recommended. Measurements of creatinine are recommended at least every 2 months.

2.1.8 Storage Conditions

Methotrexate should be kept in its original container, tightly closed, and out of reach of children. It should be stored at room temperature, away from excess heat and moisture (not in the bathroom). Any medication that is outdated or no longer needed should be disposed off. Talk to the pharmacist about the proper disposal of medication\(^\text{(92, 93, 94, and 95)}\).
2.2 DOXORUBICIN

- Molecular formula of doxorubicin is \( C_{27}H_{29}NO_{11} \)
- Chemical IUPAC Name is 10-(4-amino-5-hydroxy-6-methyl-oxan-2-yl)oxy-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-9,10-dihydro-7H-tetracene-5,12-dione
- Molecular weight is 543.519 g/mol

Doxorubicin hydrochloride (Adriamycin\textsuperscript{®}) is a prescription medication used to treat various types of cancer. This includes breast cancer, lung cancer, stomach cancer and ovarian cancer, to name a few. It is part of a group of chemotherapy medications called anthracyclines.

Doxorubicin's most serious adverse effect is life-threatening heart damage. The drug is administered intravenously, in the form of hydrochloride salt. It may be sold under the brand names Adriamycin PFS, Adriamycin RDF, or Rubex. Doxorubicin is photosensitive, and containers are often covered by an aluminum bag and/or brown wax paper to prevent light from affecting it.
The molecule was originally isolated in the 1950s from bacteria found in soil samples taken from Castel del Monte, an Italian castle.

2.2.1 Biosynthesis

Doxorubicin (DXR) is a 14-hydroxylated version of daunorubicin, the immediate precursor of DXR in its biosynthetic pathway. Daunorubicin is more abundantly found as a natural product because it is produced by a number of different wild type strains of Streptomyces.

2.2.2 Mechanism Of Action

Inhibition of DNA and RNA synthesis by intercalation between DNA base pairs by inhibition of topoisomerase II and by steric obstruction. Doxorubicin intercalates at points of local uncoiling of the double helix. Although the exact mechanism is unclear, it appears that direct binding to DNA (intercalation) and inhibition of DNA repair (topoisomerase II inhibition) result in blockade of DNA and RNA synthesis and fragmentation of DNA. Doxorubicin is also a powerful iron chelator; the iron-doxorubicin complex can bind DNA and cell membranes and produce free radicals that immediately cleave the DNA and cell membranes\(^{(96)}\).

2.2.3 Adverse Reactions

Acute adverse effects of doxorubicin can include nausea, vomiting, and heart arrhythmias. It can also cause neutropenia (a decrease in white blood cells), as well as complete alopecia (hair loss). When the cumulative dose of doxorubicin reaches 550 mg/m\(^2\), the risks of developing cardiac side effects, including CHF, dilated cardiomyopathy, and death, dramatically increase. Doxorubicin cardiotoxicity is characterized by a dose-dependent decline in mitochondrial oxidative phosphorylation. Reactive oxygen species, generated by the interaction of doxorubicin with iron, can then damage the myocytes (heart cells), causing myofibrillar loss and cytoplasmic
vacuolization. Additionally, some patients may develop PPE (palmer plantar erythrodynesesthesia), characterized by skin eruptions on the palms of the hand or soles of the feet, swelling, pain and erythema. Due to these side effects and its red color, doxorubicin has earned the nickname "red devil" or "red death." Chemotherapy can cause reactivation of hepatitis B, and doxorubicin-containing regimens are no exception (97, 98, 99 and 100).

2.2.4 Pharmacodynamics/Kinetics

Absorption: Oral: Poor (<50%)

Distribution: $V_d$: 809-1214 L/m$^2$; to many body tissues, particularly liver, spleen, kidney, lung, heart; does not distribute into the CNS; crosses placenta.

Protein binding, plasma: 70% to 76%

Metabolism: Primarily hepatic to doxorubicinol (active), then to inactive aglycones, conjugated sulfates, and glucuronides

Half-life elimination:

Distribution: 5-10 minutes

Elimination: Doxorubicin: 1-3 hours; Metabolites: 3-3.5 hours

Terminal: 17-48 hours

Male: 54 hours; Female: 35 hours

Excretion: Feces (~40% to 50% as unchanged drug); urine (~5% to 12% as unchanged drug and metabolites)

Clearance: Male: 113 L/hour; Female: 44 L/hour

2.2.5 Contraindications

Hypersensitivity to doxorubicin, any component of the formulation, or to other anthracyclines or anthracenediones; recent MI, severe myocardial insufficiency, severe
arrhythmia; previous therapy with high cumulative doses of doxorubicin, daunorubicin, idarubicin, or other anthracycline and anthracenediones; baseline neutrophil count <1500/mm³; severe hepatic impairment

2.2.6 Dosage

Children: 35-75 mg/m²/dose every 21 days or 20-30 mg/m²/dose once weekly or 60-90 mg/m²/dose given as a continuous infusion over 96 hours every 3-4 weeks

Adults: Usual or typical dose: 60-75 mg/m²/dose every 21 days or 60 mg/m²/dose every 2 weeks (dose dense) or 40-60 mg/m²/dose every 3-4 weeks or 20-30 mg/m²/day for 2-3 days every 4 weeks or 20 mg/m²/dose once weekly.

2.2.7 Dosage Forms

Excipient information presented when available (limited, particularly for generics); consult specific product labeling. Injection, powder for reconstitution, as hydrochloride: 10 mg, 50 mg, Adriamycin®: 10 mg, 20 mg, 50 mg, [contains lactose], Injection, solution, as hydrochloride: 2 mg/mL (5 mL, 10 mL, 25 mL, 100 mL), Adriamycin®: 2 mg/mL (5 mL, 10 mL, 25 mL, 100 mL).

2.2.8 Clinical Use

Doxorubicin is commonly used to treat some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Commonly used doxorubicin-containing regimens are AC (Adriamycin, cyclophosphamide), TAC (Taxotere, CA), ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), BEACOPP, CHOP (cyclophosphamide, Adriamycin, vincristine, prednisone) and FAC (5-fluorouracil, Adriamycin, cyclophosphamide). Doxil is used primarily for the treatment of ovarian
cancer where the disease has progressed or recurred after platinum-based chemotherapy, or for the treatment of AIDS-related Kaposi’s sarcoma\textsuperscript{(105, 106)}.

Experimental therapy - Combination therapy experiments with sirolimus (rapamycin) and doxorubicin have shown promise in treating Akt-positive lymphomas in mice.

Recent animal research coupling a murine monoclonal antibody with doxorubicin has created an immunoconjugate that was able to eliminate HIV-1 infection in mice. Current treatment with antiretroviral therapy (ART) still leaves pockets of HIV within the host. The immunoconjugate could potentially provide a complementary treatment to ART to eradicate antigen-expressing T cells.

Liposomal formulations - Doxil is a pegylated (polyethylene glycol coated) liposome-encapsulated form of doxorubicin made by Ben Venue Laboratories for Johnson & Johnson in the United States. It was developed to treat Kaposi’s sarcoma, an AIDS-related cancer that causes lesions to grow under the skin, in the lining of the mouth, nose and throat, or in other organs. The polyethylene glycol coating results in preferential concentration of Doxil in the skin. However, this also results in a side effect called palmar plantar erythrodysesthesia (PPE), more commonly known as hand-foot syndrome. Following administration of Doxil, small amounts of the drug can leak from capillaries in the palms of the hands and soles of the feet. The result of this leakage is redness, tenderness, and peeling of the skin that can be uncomfortable and even painful. In clinical testing at 50 mg/m\textsuperscript{2} dosing every 4 weeks, 50.6% of patients treated with Doxil developed hand-foot syndrome. The prevalence of this side effect limits the Doxil dose that can be given as compared with doxorubicin in the same treatment regimen, thereby limiting potential substitution. Substitution would be desirable because liposome-encapsulated doxorubicin is less cardiotoxic than unencapsulated doxorubicin. Doxil is also approved by the FDA for treatment of ovarian cancer and multiple
myeloma. Outside the United States, Doxil is known as Caelyx and is marketed by Janssen.

Myocet is a non-pegylated liposomal doxorubicin made by Enzon Pharmaceuticals for Cephalon in Europe and for Sopherion Therapeutics in the United States and Canada. Myocet is approved in Europe and Canada for treatment of metastatic breast cancer in combination with cyclophosphamide, but is not yet approved by the FDA for use in the United States. It is currently being studied by Sopherion Therapeutics in a pivotal phase III global registrational trial in concurrent combination with trastuzumab (Herceptin) and paclitaxel (Taxol) for treatment of HER2-positive metastatic breast cancer. Unlike Doxil, the Myocet liposome does not have a polyethylene glycol coating, and therefore does not result in the same prevalence of hand-foot syndrome. The minimization of this side effect may allow for one for one substitution with doxorubicin in the same treatment regimen, thereby improving safety with no loss of efficacy. Like Doxil, the liposomal encapsulation of the doxorubicin limits the cardiotoxicity. In theory, by limiting the cardiotoxicity of doxorubicin through liposomal encapsulation, it can be used safely in concurrent combination with other cardiotoxic chemotherapy drugs, such as trastuzumab. There is an FDA black box warning that Herceptin cannot be used in concurrent combination with doxorubicin, only in sequential combination. Though concurrent combination of trastuzumab and doxorubicin in clinical studies found superior tumor response, the combination resulted in unacceptable cardiotoxicity, including risk of cardiac failure manifesting as congestive heart failure (CHF). Published phase II study results have shown that Myocet, trastuzumab, and paclitaxel can safely be used concurrently without the cardiac risk, as measured by reduction in LVEF function, while still achieving superior tumor response. This finding is the basis for the on-going phase III trial for FDA approval\(^{(95)}\).
2.2.9 Storage

Doxorubicin should be stored at room temperature in clearly labeled, tightly closed containers within a designated area. It should be kept away from direct sunlight or strong incandescent light. Keep away from heat/flame and moisture.
2.3 5-FLUOROURACIL

- Molecular Weight: 130.08
- IUPAC Nomenclature: 2, 4-Dihydroxy-5-fluoropyrimidine; 5-Fluoro-2,4(1H,3H)-pyrimidinedione.
- Molecular Formula: C$_4$H$_3$FN$_2$O$_2$

2.3.1 Definition

Fluorouracil (5-FU or f5U) (sold under the brand names Adrucil, Carac, Efudix, Efudex and Fluoroplex) is a drug that is a pyrimidine analog that interferes with the growth of cancer cells so it is used in the treatment of cancer. It is a suicide inhibitor and works through irreversible inhibition of thymidylate synthase. It belongs to the family of drugs called antimetabolites. It can be used to treat many types of cancers, including cancer of the colon, rectum, breast, stomach, head, and neck.

2.3.2 Synthesis

5-FU was designed, synthesized and patented by Charles Heidelberger in 1957. Since uracil is a normal component of RNA, the rationale behind the development of the drug was that cancer cells, with their increased genetic instability, might be more sensitive to 'decoy' molecules that mimic the natural compound than normal cells. The scientific goal
in this case was to synthesize a drug which demonstrated specific uracil antagonism. The drug proved to have anti-tumor capabilities. When elemental fluorine is reacted with uracil, 5-fluorouracil is produced. 5-Fluorouracil masquerades as uracil during the nucleic acid replication process. Because 5-Fluorouracil is similar in shape to, but does not perform the same chemistry as uracil the drug inhibits RNA replication enzymes, thereby eliminating RNA synthesis and stopping the growth of cancerous cells\(^{95, 107, \text{and} 108}\).

2.3.3 Mode Of Action

As a pyrimidine analogue, it is transformed inside the cell into different cytotoxic metabolites which are then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell’s ability to synthesize DNA. It is an S-phase specific drug and only active during certain cell cycles. In addition to being incorporated in DNA and RNA, the drug has been shown to inhibit the activity of the exosome complex, an exoribonuclease complex of which the activity is essential for cell survival. Capecitabine is a prodrug that is converted into 5-FU in the tissues. It can be administered orally.

2.3.4 Side Effects

5-FU is normally administered by intravenous route. The life span of 5-FU in blood and body tissues is very short and limited to minutes. 5-FU binds to an enzyme inside the cancer cells called thymidilate synthetase and thereby exert its anti cancer effect on the cells. Leucovorin enhances the binding of 5-FU to this enzyme and as a result prolongs the life span of 5-FU within the cancer cells, resulting in a greater anti cancer effect. The degree and severity of the side effects depend on the amount and schedule of the administration of 5-FU. Some patients have deficiency of an enzyme (DPD Deficiency)
that is crucial for the metabolism and deactivation of 5-FU. Such patients suffer from severe side effects with smallest doses and often the very first dose of 5-FU \(^{109, 110}\).

Following are some of the most common and important ill effects:

- Soreness of the mouth,
- difficulty swallowing
- Diarrhea, with Stomach pain
- Low white blood counts
- Low platelet counts
- Anemia
- Sensitive skin to sun exposure
- Excessive tear formation

### 2.3.5 Uses

The chemotherapy agent 5-FU (fluorouracil), which has been in use against cancer for about 40 years, acts in several ways, but principally as a thymidylate synthetase inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleotide required for DNA replication. Thymidylate synthetase methylates deoxyuridine monophosphate (dUMP) into thymidine monophosphate (dTMP). Facing a scarcity of dTMP, rapidly dividing cancerous cells undergo cell death via thymineless death.

Like many anti-cancer drugs, 5-FU's effects are felt system wide but fall most heavily upon rapidly dividing cells that make heavy use of their nucleotide synthesis machinery, such as cancer cells (other parts of the body with rapidly dividing cells include the cells lining the digestive tract).
Some of its principal uses are in colorectal cancer, and pancreatic cancer, in which it has been the established form of chemotherapy for decades (platinum-containing drugs approved for human use in the US since 1978 are also very well established). It is also sometimes used in the treatment of inflammatory breast cancer, an especially aggressive form of breast cancer.

5-FU is also used in ophthalmic surgery, specifically to augment trabeculectomy (an operation performed to lower the intraocular pressure in patients with glaucoma) in patients deemed to be at high risk for failure. 5-FU acts as an anti-scarring agent in this regard, since excessive scarring at the trabeculectomy site is the main cause for failure of the surgery.

Fluorouracil can be used topically (as a cream) for treating actinic (solar) keratoses and some types of basal cell carcinomas of the skin. It is often referred to by its trade names Efudex, Carac or Fluoroplex.

Due to Fluorouracil's toxicity and the fact that it can be manufactured using the same reaction as uracil, its precursor, 5-Fluoroorotic Acid, is commonly used in laboratories to screen against organisms capable of synthesizing uracil. It is a key component in Tegafur-uracil.

2.3.6 Clinical Pharmacology

There is evidence that the metabolism of fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid. In this manner fluorouracil interferes with the synthesis of deoxyribonucleic acid (DNA) and to a lesser extent inhibits the formation of ribonucleic acid (RNA). Since DNA and RNA are essential for cell division and growth, the effect of fluorouracil may be to create a thymine deficiency which provokes unbalanced growth and death of the cell. The effects
of DNA and RNA deprivation are most marked on those cells which grow more rapidly and take up fluorouracil at a more rapid rate. The catabolic metabolism of fluorouracil results in degradation products (e.g., CO₂, urea, α-fluoro-β-alanine) which are inactive.

Systemic absorption studies of topically applied fluorouracil have been performed on patients with actinic keratoses using tracer amounts of ¹⁴C-labeled fluorouracil added to a 5% preparation. All patients had been receiving nonlabeled fluorouracil until the peak of the inflammatory reaction occurred (2 to 3 weeks), ensuring that the time of maximum absorption was used for measurement. One gram of labeled preparation was applied to the entire face and neck and left in place for 12 hours. Urine samples were collected. At the end of 3 days, the total recovery ranged between 0.48% and 0.94% with an average of 0.76%, indicating that approximately 5.98% of the topical dose was absorbed systemically. If applied twice daily, this would indicate systemic absorption of topical fluorouracil to be in the range of 5 to 6 mg per daily dose of 100 mg. In an additional study, negligible amounts of labeled material were found in plasma, urine and expired CO₂ after 3 days of treatment with topically applied ¹⁴C-labeled fluorouracil.

2.3.7 Indications And Usage

Fluorouracil is recommended for the topical treatment of multiple actinic or solar keratoses. In the 5% strength it is also useful in the treatment of superficial basal cell carcinomas when conventional methods are impractical, such as with multiple lesions or difficult treatment sites. Safety and efficacy in other indications have not been established.

The diagnosis should be established prior to treatment, since this method has not been proven effective in other types of basal cell carcinomas. With isolated, easily accessible basal cell carcinomas, surgery is preferred since success with such lesions is almost
100%. The success rate with fluorouracil cream and solution is approximately 93%, based on 113 lesions in 54 patients. Twenty-five lesions treated with the solution produced 1 failure and 88 lesions treated with the cream produced 7 failures.

2.3.8 Contraindication

Fluorouracil may cause fetal harm when administered to a pregnant woman.

There are no adequate and well-controlled studies in pregnant women with either the topical or the parenteral forms of fluorouracil. One birth defect (cleft lip and palate) has been reported in the newborn of a patient using fluorouracil as recommended. One birth defect (ventricular septal defect) and cases of miscarriage have been reported when Fluorouracil was applied to mucous membrane areas. Multiple birth defects have been reported in a fetus of a patient treated with intravenous fluorouracil.

Animal reproduction studies have not been conducted with fluorouracil. Fluorouracil administered parenterally has been shown to be teratogenic in mice, rats, and hamsters when given at doses equivalent to the usual human intravenous dose; however, the amount of fluorouracil absorbed systemically after topical administration to actinic keratoses is minimal. Fluorouracil exhibited maximum teratogenicity when given to mice as single intraperitoneal injections of 10 to 40 mg/kg on Day 10 or 12 of gestation. Similarly, intraperitoneal doses of 12 to 37 mg/kg given to rats between Days 9 and 12 of gestation and intramuscular doses of 3 to 9 mg/kg given to hamsters between Days 8 and 11 of gestation were teratogenic and/or embryotoxic (ie, resulted in increased resorptions or embryolethality). In monkeys, divided doses of 40 mg/kg given between Days 20 and 24 of gestation were not teratogenic. Doses higher than 40 mg/kg resulted in abortion.

Fluorouracil should not be used in patients with dihydropyrimidine dehydrogenase (DPD) enzyme deficiency. A large percentage of fluorouracil is catabolized by the DPD
enzyme. DPD enzyme deficiency can result in shunting of fluorouracil to the anabolic pathway, leading to cytotoxic activity and potential toxicities.

Fluorouracil is contraindicated in women who are or may become pregnant during therapy. If this drug is used during pregnancy, or if the patient becomes pregnant while using this drug, the patient should be apprised of the potential hazard to the fetus.

Fluorouracil is also contraindicated in patients with known hypersensitivity to any of its components.

2.3.9 Warnings

Application to mucous membranes should be avoided due to the possibility of local inflammation and ulceration. Additionally, cases of miscarriage and a birth defect (ventricular septal defect) have been reported when fluorouracil was applied to mucous membrane areas during pregnancy.

Occlusion of the skin with resultant hydration has been shown to increase percutaneous penetration of several topical preparations. If any occlusive dressing is used in treatment of basal cell carcinoma, there may be an increase in the severity of inflammatory reactions in the adjacent normal skin. A porous gauze dressing may be applied for cosmetic reasons without increase in reaction.

Exposure to ultraviolet rays should be minimized during and immediately following treatment with fluorouracil because the intensity of the reaction may be increased.

Patients should discontinue therapy with fluorouracil if symptoms of DPD enzyme deficiency develop.
Rarely, life-threatening toxicities such as stomatitis, diarrhea, neutropenia, and neurotoxicity have been reported with intravenous administration of fluorouracil in patients with DPD enzyme deficiency. One case of life-threatening systemic toxicity has been reported with the topical use of Fluorouracil in a patient with DPD enzyme deficiency. Symptoms included severe abdominal pain, bloody diarrhea, vomiting, fever, and chills. Physical examination revealed stomatitis, erythematous skin rash, neutropenia, thrombocytopenia, inflammation of the esophagus, stomach, and small bowel. Although this case was observed with 5% fluorouracil cream, it is unknown whether patients with profound DPD enzyme deficiency would develop systemic toxicity with lower concentrations of topically applied fluorouracil.

2.3.10 Adverse Reactions

The most frequent adverse reactions to Fluorouracil occur locally and are often related to an extension of the pharmacological activity of the drug. These include burning, crusting, allergic contact dermatitis, erosions, erythema, hyperpigmentation, irritation, pain, photosensitivity, pruritus, scarring, rash, soreness and ulceration. Ulcerations, other local reactions, cases of miscarriage and a birth defect (ventricular septal defect) have been reported when fluorouracil was applied to mucous membrane areas. Leukocytosis is the most frequent hematological side effect.

Although a causal relationship is remote, other adverse reactions which have been reported infrequently are:

Central Nervous System: Emotional upset, insomnia, irritability.

Gastrointestinal: Medicinal taste, stomatitis.

Hematological: Eosinophilia, thrombocytopenia, toxic granulation.
Integumentary: Alopecia, blistering, bullous pemphigoid, discomfort, ichthyosis, scaling, suppuration, swelling, telangiectasia, tenderness, urticaria, skin rash.

Special Senses: Conjunctival reaction, corneal reaction, lacrimation, nasal irritation.

Miscellaneous: Herpes simplex.

2.3.11 Over dosage

There have been no reports of overdosage with fluorouracil.

The oral LD$_{50}$ for the 5% topical cream was 234 mg/kg in rats and 39 mg/kg in dogs. These doses represented 11.7 and 1.95 mg/kg of fluorouracil, respectively. Studies with a 5% topical solution yielded an oral LD$_{50}$ of 214 mg/kg in rats and 28.5 mg/kg in dogs, corresponding to 10.7 and 1.43 mg/kg of fluorouracil, respectively. The topical application of the 5% cream to rats yielded an LD$_{50}$ of greater than 500 mg/kg.

2.3.12 Dosage and Administration

When fluorouracil is applied to a lesion, a response occurs with the following sequence: erythema, usually followed by vesiculation, desquamation, erosion and reepithelialization.

Fluorouracil should be applied preferably with a nonmetal applicator or suitable glove. If fluorouracil is applied with the fingers, the hands should be washed immediately afterward.

**Actinic or Solar Keratosis**

Apply cream twice daily in an amount sufficient to cover the lesions. Medication should be continued until the inflammatory response reaches the erosion stage, at which time
use of the drug should be terminated. The usual duration of therapy is from 2 to 4 weeks. Complete healing of the lesions may not be evident for 1 to 2 months following cessation of fluorouracil therapy.

**Superficial Basal Cell Carcinomas**

Only the 5% strength is recommended. Apply cream twice daily in an amount sufficient to cover the lesions. Treatment should be continued for at least 3 to 6 weeks. Therapy may be required for as long as 10 to 12 weeks before the lesions are obliterated. As in any neoplastic condition, the patient should be followed for a reasonable period of time to determine if a cure has been obtained\(^{(95)}\).

**2.3.13 Storage**

It should be stored in tightly closed container. It should be kept in a cool, well-ventilated area and should not store above 4°C (39.2°F).
2.4 PECTIN

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and used as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, medicines, as a stabilizer in fruit juices and milk drinks, and as a source of dietary fiber.

2.4.1 Biology

Plant cells, pectin consists of a complex set of polysaccharides that are present in most primary cell walls and are particularly abundant in the non-woody parts of terrestrial plants. Pectin is present not only throughout primary cell walls but also in the middle lamella between plant cells, where it helps to bind cells together.

The amount, structure and chemical composition of pectin differs among plants, within a plant over time, and in various parts of a plant. Pectin is an important cell wall polysaccharide that allows primary cell wall extension and plant growth. During fruit ripening, pectin is broken down by the enzymes pectinase and pectinesterase, in
which process the fruit becomes softer as the middle lamellae break down and cells become separated from each other. A similar process of cell separation caused by the breakdown of pectin occurs in the abscission zone of the petioles of deciduous plants at leaf fall.

Pectin is a natural part of the human diet, but does not contribute significantly to nutrition. The daily intake of pectin from fruits and vegetables can be estimated to be around 5 g (assuming consumption of approximately 500 g fruits and vegetables per day).

In human digestion, pectin binds to cholesterol in the gastrointestinal tract and slows glucose absorption by trapping carbohydrates. Pectin is thus a soluble dietary fiber. Consumption of pectin has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health (prebiotic effect).

2.4.2 Chemistry

Pectins, also known as pectic polysaccharides, are rich in galacturonic acid. Several distinct polysaccharides have been identified and characterised within the pectic group. Homogalacturonans are linear chains of α-(1-4)-linked D-galacturonic acid. Substituted galacturonans are characterized by the presence of saccharide appendant residues (such as D-xylose or D-apiose in the respective cases of xylogalacturonan and apiogalacturonan) branching from a backbone of D-galacturonic acid residues. Rhamnogalacturonan I pectins (RG-I) contain a backbone of the repeating disaccharide: 4)-α-D-galacturonic acid-(1, 2)-α-L-rhamnose-(1). From many of the rhamnose residues,
side chains of various neutral sugars branch off. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, with the types and proportions of neutral sugars varying with the origin of pectin.

Another structural type of pectin is rhamnogalacturonan II (RG-II), which is a less frequent complex, highly branched polysaccharide. Rhamnogalacturonan II is classified by some authors within the group of substituted galacturonans since the rhamnogalacturonan II backbone is made exclusively of D-galacturonic acid units.

Isolated pectin has a molecular weight of typically 60–130,000 g/mol, varying with origin and extraction conditions. In nature, around 80 percent of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased to a varying degree during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. This is why pectins are classified as high- vs. low-ester pectins (short HM vs. LM-pectins), with more or less than half of all the galacturonic acid esterified.

The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium, or calcium. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5 percent the salts are called pectates, the insoluble acid form, pectic acid.

Some plants such as sugar beet, potatoes and pears contain pectins with acetylated galacturonic acid in addition to methyl esters. Acetylation prevents gel-formation but increases the stabilising and emulsifying effects of pectin.

Amidated pectin is a modified form of pectin. Here, some of the galacturonic acid is converted with ammonia to carboxylic acid amide. These pectins are more tolerant of varying calcium concentrations that occur in use.
To prepare a pectin-gel, the ingredients are heated, dissolving the pectin. Upon cooling below gelling temperature, a gel starts to form. If gel formation is too strong, syneresis or a granular texture are the result, whilst weak gelling leads to excessively soft gels. In high-ester pectins at soluble solids content above 60% and a pH-value between 2.8 and 3.6, hydrogen bonds and hydrophobic interactions bind the individual pectin chains together. These bonds form as water is bound by sugar and forces pectin strands to stick together. These form a 3-dimensional molecular net that creates the macromolecular gel. The gelling-mechanism is called a low-water-activity gel or sugar-acid-pectin gel.

In low-ester pectins, ionic bridges are formed between calcium ions and the ionised carboxyl groups of the galacturonic acid. This is idealised in the so-called “egg box-model”. Low-ester pectins need calcium to form a gel, but can do so at lower soluble solids and higher pH-values than high-ester pectins.

Amidated pectins behave like low-ester pectins but need less calcium and are more tolerant of excess calcium. Also, gels from amidated pectin are thermo-reversible; they can be heated and after cooling solidify again, whereas conventional pectin-gels will afterwards remain liquid.

High-ester pectins set at higher temperatures than low-ester pectins. However, gelling reactions with calcium increase as the degree of esterification falls. Similarly, lower pH-values or higher soluble solids (normally sugars) increase gelling speed. Suitable pectins can therefore be selected for jams and for jellies, or for higher sugar confectionery jellies.

### 2.4.3 Sources and production

Apples, guavas, quince, plums, gooseberries, oranges and other citrus fruits, contain large amounts of pectin, while soft fruits like cherries, grapes and strawberries contain small amounts of pectin. Typical levels of pectin in plants are (fresh weight):
- apples, 1–1.5%
- apricot, 1%
- cherries, 0.4%
- oranges, 0.5–3.5%
- carrots approx. 1.4%
- citrus peels, 30%

The main raw-materials for pectin production are dried citrus peel or apple pomace, both by-products of juice production. Pomace from sugar-beet is also used to a small extent.

From these materials, pectin is extracted by adding hot dilute acid at pH-values from 1.5 – 3.5. During several hours of extraction, the protopectin loses some of its branching and chain-length and goes into solution. After filtering, the extract is concentrated in vacuum and the pectin then precipitated by adding ethanol or isopropanol. An old technique of precipitating pectin with aluminium salts is no longer used (apart from alcohols and polyvalent cations; pectin also precipitates with proteins and detergents).

Alcohol-precipitated pectin is then separated, washed and dried. Treating the initial pectin with dilute acid leads to low-esterified pectins. When this process includes ammonium hydroxide, amidated pectins are obtained. After drying and milling, pectin is usually standardised with sugar and sometimes calcium-salts or organic acids to have optimum performance in a particular application. Worldwide, approximately 40,000 metric tons of pectin is produced every year.

**2.4.4 Uses**

The main use for pectin (vegetable agglutinate) is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in gelling sugar (also known as "jam sugar") where it is diluted to the
right concentration with sugar and some citric acid to adjust pH. In some countries, pectin is also available as a solution or an extract, or as a blended powder, for home jam making. For conventional jams and marmalades that contain above 60% sugar and soluble fruit solids, high-ester pectins are used. With low-ester pectins and amidated pectins less sugar is needed, so that diet products can be made. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked foods. Typical levels of pectin used as a food additive are between 0.5 – 1.0% - this is about the same amount of pectin as in fresh fruit. In medicine, pectin increases viscosity and volume of stool so that it is used against constipation and diarrhoea. Until 2002, it was one of the main ingredients used in Kaopectate a drug to combat diarrhoea, along with kaolinite. Pectin is also used in throat lozenges as a demulcent. In cosmetic products, pectin acts as stabilizer. Pectin is also used in wound healing preparations and specialty medical adhesives, such as colostomy devices. Also, it is considered a natural remedy for nausea. Pectin rich foods are proven to help nausea. In ruminant nutrition, depending on the extent of lignification of the cell wall, pectin is up to 90% digestible by bacterial enzymes. Ruminant nutritionists recommend that the digestibility and energy concentration in forages can be improved by increasing pectin concentration in the forage. In the cigar industry, pectin is considered an excellent substitute for vegetable glue and many cigar smokers and collectors will use pectin for repairing damaged tobacco wrapper leaves on their cigars. Pectin is also used in jellybeans.

2.4.5 Legal Status

At the FAO/WHO joint Expert Committee on Food Additives and in the EU, no numerical acceptable daily intake (ADI) has been set, as pectin is considered safe. In the US, pectin is in GRAS – generally recognized as safe category. In most foods it can be used according to good manufacturing practices in the levels needed for its application,
"quantum satis". In the International Numbering System (INS), pectin has the number 440. In Europe, pectins are differentiated into the E numbers E440 (i) for non-amidated pectins and E440 (ii) for amidated pectins. There are specifications in all national and international legislation defining its quality and regulating its use\(^{(95)}\).
Casein is a protein that is found in milk and used independently in many foods as a binding agent. Technically, it is part of a group called phosphoproteins, collections of proteins bound to something containing phosphoric acid. Casein may also be called caseinogen, particularly in European foods. Casein is a salt, meaning it has no net ionic charge, of the element calcium. It has a number of interesting properties that make it useful in foods and cooking. Many people believe proteins are healthier if consumed when not denatured – one of the major lines of reasoning used in supporting a raw food diet. Denaturation occurs when a protein loses its inherent structure, due to high heat or acid for example, at which point it no longer acts in the ordinary manner. Casein, because of its structure, is not susceptible to denaturing. Casein can be found in two main types: edible and technical. Edible casein is widely used in both medicine and food, both for nutritional value and as a binder. Technical casein is used in an enormous range of products, including paints, cosmetics, and many types of adhesives. A substantial number of people have a casein allergy and may find themselves experiencing negative reactions both to casein-containing food products and to products such as nail polish that contain casein\(^{(95)}\).