Chapter II

Drug Profile

2.1 Doxorubicin Hydrochloride
2.2 5-Fluorouracil
2.3 Results and Discussion
Brain carcinoma is a life threatening disease and limited treatments are available at present. This could be due to covering of the brain with highly lipophilic layer, BBB that restricts the entry of the anticancer drugs into brain. The two anticancer drugs, doxorubicin HCl and 5-Fluorouracil were selected to develop drug delivery systems that would deliver drugs in effective concentration to the brain by utilizing either receptor mediated transcytosis mechanism or carrier mediated transport system.

2.1 DOXORUBICIN HYDROCHLORIDE

Doxorubicin is an antineoplastic antibiotic isolated from a culture of *Streptomyces peucetius* var. *coesius* or by chemical synthesis from daunorubicin. The injectable dosage form is supplied as the hydrochloride salt in combination with lactose as a freeze-dried powder (USP, EP, IP)

**Structure**

![Chemical Structure of Doxorubicin Hydrochloride](image)

**Chemical name** (85-105)-10-(3-amino-2,3,6-trideoxy-α-L-lyxohexopyranosyl) oxy 7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-hydroxyacetyl-1-methoxy-5,12-naphthacenedione
Molecular Formula C_{27}H_{29}NO_{11} and its hydrochloride salt

Pharmacopoeial Status USP, EP and IP

Category Antineoplastic antibiotic

Appearance, Color, Odor The hydrochloride salt is a red, free-flowing crystalline powder, odorless

Melting Point Doxorubicin HCl melts at 205°C with decomposition.

Optical Rotation The optical rotation of doxorubicin HCl in methanol (0.1%) is +255°.

Ionization Constant Doxorubicin HCl shows indicator-like properties, turning from orange-red to blue-violet at about pH 9.0 and it's pKa value is 8.22 (Sturgeon and Schulman, 1977)

Solubility Doxorubicin HCl is readily soluble in water, normal saline, methanol, acetonitrile and tetrahydrofuran and practically insoluble in chloroform, ether and other organic solvents.

Partition Coefficient The partition coefficient between 1-octanol and tris buffer at pH 7.0 with constant ionic strength (I=0.1) is 0.52 at room temperature (22-24°C)

Storage Doxorubicin HCl should be stored in well-closed, airtight containers.

Stability Doxorubicin HCl is very stable in the solid state and active drug substance has also been found to be stable for three months at 60 °C. The lyophilized powder of doxorubicin HCl with lactose is also stable, if dry and stored in well closed
containers at room temperature (Arcamone et al., 1972). Doxorubicin HCl is stable at the pH range of 3.0 to 6.5, but decomposes at increasing rates as the pH increases from 6.5 to 12.0. In solution form, it is photodegradable and loses activity if not protected from light properly.

2.1.1 Pharmacology

2.1.1.1 Action and uses
Doxorubicin has the widest antineoplastic spectrum. It binds to DNA and inhibits nucleic acid synthesis, inhibits mitosis and promotes chromosomal aberrations. It is the drug of first choice for the treatment of thyroid acenoma and primary hepatocellular carcinoma. It is also used for the treatment of ovarian, breast, endometrial tumors, non small cell lung carcinoma, gastric adenoma carcinoma, retinoblastinoma, prostatic carcinoma, bladder carcinoma, nilms tumor, Hodgkin's disease and soft tissue carcinoma. It is also used in the treatment of acute lymphocytic leukaemia, acute myeloblastic leukemia, bladder cancer, breast cancer, endometrial cancer, gastric cancer, head and neck cancer, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, ovarian cancer, sarcoma soft tissue, testicular cancer, thyroid cancer, Wilms' tumour, adrenocortical cancer, carcinoid syndrome (small bowel), Ewing's sarcoma, gynecological sarcoma, hepatic cancer, islet cell cancer, multiple myeloma, pancreatic cancer, and rhabdomyosarcoma.

2.1.1.2 Mechanism of action
Doxorubicin, is an anthracycline antibiotic produced by the fungus streptomyces peucetius. Doxorubicin damages DNA by intercalation of the anthracycline portion, metal ion chelation, or by generation of free radicals. Doxorubicin has also been shown to inhibit DNA topoisomerase II which is critical to DNA function (Haskell, 1990; Chabner et al., 1989; Dorr et al., 1980).
2.1.1.3 Dose and dosage forms

**Adults**

- intravenous: 60-90 mg/kg and 20-30 mg/kg body weight
- intra-arterial: 25 mg/kg body weight
- intravesical: 40-80 mg/kg body weight

**Dosage Forms** (Dorr et al., 1980; Knoben et al., 1984; Reynolds, 1982; McEvoy, 1991; Pagliaro and Pagliaro, 1987; Torti, 1984; Rapp et al., 1984)

<table>
<thead>
<tr>
<th>Route</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>Not used due to corrosive nature</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Not used due to corrosive nature</td>
</tr>
<tr>
<td>Direct</td>
<td>Preferred method due to need for frequent monitoring for signs of extravasation. Via small (21 or 23) gauge needle into tubing of running IV. Push slowly, so that drip of IV solution does not stop or reverse. Check for blood return before administration and after every 2-3 ml of drug. If no blood returns, stop the injection and assess the IV site. Flush with 20 ml normal saline or distilled water after administration to clear any remaining drug from tubing.</td>
</tr>
<tr>
<td>Intermittent</td>
<td>In 100 ml over 1 hour, central venous access preferred</td>
</tr>
<tr>
<td>Continuous</td>
<td>As per protocol or dilute in a convenient volume, central venous access only</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Not recommended due to risk of chemical peritonitis, has been done as investigational procedure.</td>
</tr>
<tr>
<td>Intrapleural</td>
<td>Not recommended, has been used in treatment of malignant effusions</td>
</tr>
<tr>
<td>Intrathecal</td>
<td>Not used due to corrosive nature</td>
</tr>
<tr>
<td>Intra-arterial</td>
<td>Investigational continuous infusion into unresectable tumour mass via radiographically placed cannula</td>
</tr>
<tr>
<td>Intravesical</td>
<td>In 50-100 ml normal saline, retained 1-2 hours</td>
</tr>
</tbody>
</table>
Maximum Lifetime Dose

Adults and children: 300-450 mg/kg depending on risk factors

2.1.1.4 Pharmacokinetic parameters

It is poorly absorbed by the gastrointestinal route that is why doxorubicin is most commonly administered intravenously. The pharmacokinetic parameters of doxorubicin are recoded in Table 2.1.

<table>
<thead>
<tr>
<th>Oral Absorption</th>
<th>No significant absorption through the gastrointestinal tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Highest concentration is found in liver, spleen, kidneys, heart, small intestine, lungs, placenta, and breast milk.</td>
</tr>
<tr>
<td>Vd</td>
<td>25 l/kg</td>
</tr>
<tr>
<td>PPB</td>
<td>79-85%</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Liver &amp; other tissues; elimination primarily via liver and biliary system</td>
</tr>
<tr>
<td>Active metabolite(s)</td>
<td>doxorubicinol (major metabolite)</td>
</tr>
<tr>
<td>Excretion</td>
<td>Predominantly in bile, 40-50% in feces within 7 days</td>
</tr>
<tr>
<td>Urine</td>
<td>4-5% over 5 days</td>
</tr>
<tr>
<td>t½ α</td>
<td>12 minutes</td>
</tr>
<tr>
<td>t½ β</td>
<td>3.3 hours</td>
</tr>
<tr>
<td>t½ γ</td>
<td>29.6 hours</td>
</tr>
<tr>
<td>Cl</td>
<td>17±3ml/minutes/kg, 503ml/minutes/kg (higher in children)</td>
</tr>
</tbody>
</table>

Doxorubicin is rapidly cleared from blood and distributed into tissue including lungs, liver, heart, spleen and kidneys after intravenous administration. It undergoes rapid metabolism in the liver and there are two major metabolic transformations of doxorubicin.

(a) The reduction of the side chain carbon carbonyl group to a secondary alcohol, giving 13-dihydrodoxorubicin (adriamycinol).

(b) The reductive cleavage of the daunosamine moiety with the formation of 10-deoxyadriamycinone.
About 40 to 50% of dose is excreted in bile within 7 days, of which about half is unchanged drug. Only about 5% of a dose is excreted in urine within 5 days. It does not cross the blood brain barrier but crosses the placenta. The binding of the doxorubicin with plasma and proteins is very rapid and the highest concentration is located in the liver. The hepatobiliary system is the primary route of excretion. In the study of the hepatic artery infusion, 50% of the dose of doxorubicin could be extracted in the single pass through the liver. Its elimination is triphasic.

2.1.1.5 Adverse effects

Cardiotoxicity

Therapeutic potential of doxorubicin is limited by the development of the cardiac failure. Cardiotoxicity can be divided into an initial acute effect with transient electrocardiographic abnormalities and a later cumulative, dose-dependent cardiomyopathy. The acute electrocardiographic changes are usually reversible, unrelated to total dose, return to baseline within a few days to two months and usually are not an indication to discontinue the doxorubicin. The more serious cardiotoxicity is the dose-dependent cardiomyopathy. The onset of cardiomyopathy may be delayed, occurring 6 months or more after therapy.

Hyperuricemia

During periods of active cell lysis, this is caused by cytotoxic chemotherapy of highly proliferative tumors of massive burden (eg, some leukemias and lymphomas).

Dermatological effects

Facial flushing, alopecia and hyperpigmentation of fingers.

Gastrointestinal

Stomatitis, diarrhea, nausea and vomiting. Doxorubicin may enhance the toxic effect of the radiotherapy when given in combination.
Hematologic
Doxorubicin causes bone-marrow depression with leucopenia at a maximum 10 to 15 days after administration and blood counts recover about after 21 days.

Hypersensitivity
There is a development of hypersensitivity reaction following the administration of doxorubicin. Reactions were limited to urticarial lesions and localized pruritus, change in blood pressure were mild to moderate and other effects are type I (anaphylactic), skin rash, fever and chills.

Liver
Doxorubicin causes hepatitis and non-specific hepatocellular damage. A characteristic hepatotoxicity can be produced by the combination of radiotherapy with doxorubicin.

Nervous system
Doxorubicin is neurotoxic when perfused through the cerebrospinal spaces. Its intrathecal use is not recommended.

2.1.1.6 Precautions (Reynolds, 1982; McEvoy, 1991; Perry and Yarbro, 1984; Von Hoff, 1979; Merrill et al., 1975; Minow et al., 1977; Lewis, 1981)

Cardiac toxicity
Cardiac toxicity is cumulative across members of the anthracycline (doxorubicin, epirubicin, daunorubicin) and anthracenedione (mitoxantrone) class of drugs. Patients who have received these agents are at increased risk of toxicity, and should be carefully monitored. The cumulative doses are lower in patients who have received radiation to the mediastinal area or concomitant therapy with other cardiotoxic agents such as cyclophosphamide.

Contraindication
In patients with severe cardiovascular disease, unstable conditions including hypertension, angina, arrhythmias and hyperbilirubinemia.
Doxorubicin has been shown to have mutagenic and carcinogenic properties in experimental models. Its safe use in pregnancy and its effects on fertility have not been established. The drug is present in breast milk, therefore breast feeding is not recommended.

2.1.1.7 Interactions (Reynolds, 1982; McEvoy, 1991; Hansten, 1995)
Side effects due to doxorubicin are increased when doxorubicin is given in combination with other drugs as given in the Table 2.2.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbiturates</td>
<td>Therapeutic effects of doxorubicin decreased</td>
<td>Increased plasma clearance of doxorubicin</td>
<td>Monitor if barbiturates initiated or discontinued</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Exacerbation of cyclophosphamide induced hemorrhagic cystitis</td>
<td>Uncertain</td>
<td>Caution</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Increased risk potential for cardiotoxicity</td>
<td>Uncertain</td>
<td>Monitor, may need to modify dose of doxorubicin</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Decreased digoxin levels; interaction may occur several days after treatment</td>
<td>Decreased digoxin absorption</td>
<td>Monitor digoxin levels and patient</td>
</tr>
<tr>
<td>Mercaptopurine</td>
<td>Enhanced hepatotoxicity</td>
<td>Uncertain</td>
<td>Monitor</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Antimicrobial effect of quinolones decreased</td>
<td>Decreased quinolones absorption</td>
<td>Monitor, may need to modify dose of quinolones</td>
</tr>
<tr>
<td>Streptozocin</td>
<td>Increased toxicity of doxorubicin</td>
<td>Liver damage by streptozocin decreases metabolism of doxorubicin</td>
<td>Caution</td>
</tr>
</tbody>
</table>
2.2 5-FLUOROURACIL

Structure

Chemical names  5- Fluoro-2, 4 (1H, 3H) – pyrimidinedione,  
2,4-Dioxo-5-fluoro pyrimidine

Molecular Formula  C₄H₃FN₂O₂

Molecular Weight  130.08

Pharmacopoeial  IP, EP, USP and BP

Status  Antimetabolite antineoplastic

Appearance, Color, Odor  White to practically white, odorless, crystalline powder (Rudy and Senkowski, 1973), crystals from water or methanol (Merk Index, 1983).

Melting Point  Melting point lies between 282 to 283°C with decomposition (USP).

pH  1% solution has pH of 4.3-5.3

Solubility  Soluble in water, acetone, methanol, slightly soluble in ethanol and practically insoluble in chloroform and ether.

Storage  5-fluorouracil should be stored in well closed, air tight containers, as well as protected from light.

Stability  5-fluorouracil is stable at the room temperature. When fluorouracil was dissolved in glucose
injection (5%) and it was stored in polyvinyl chloride bags, there was a 10% loss of drug from solution in 45 hours and some amount looses when stored in glass.

2.2.1 Pharmacology

2.2.1.1 Action and uses

5-fluorouracil has been used in the treatment of cancer for more than two decades. It is a fluorinated antimetabolite of pyrimidinc uracil. It slows tumour cell growth by inhibiting thymidine formation; thereby inhibits protein synthesis by incorporating into RNA.

5-fluorouracil is used alone or in combination in the palliation of inoperable malignant neoplasm especially those of the gastro-intestinal tract, breast, liver, genital-urinary system and pancreas malignancy. It is often used in combination with cyclophosphamide and methotrexate in the combination chemotherapy of breast cancer.

5-fluorouracil is used topically in the treatment of solar or actinic keratoses and other tumors and premalignant conditions of the skin including Bowen’s disease and superficial basal cell carcinomas (Hansten and Horn, 1992; Tatro, 1992).

2.2.1.2 Mechanism of action

5-fluorouracil was developed in 1957 based on the observation that tumor cells utilized the base pair uracil for DNA synthesis more efficiently than did normal cells of the intestinal mucosa. It is a fluorinated pyrimidine that is metabolized intracellularly to its active form, fluorodeoxyuridine monophosphate (FdUMP). This active form, FdUMP inhibits DNA synthesis by blocking the conversion of deoxyuridylic acid to thymidylic acid by the cellular enzyme thymidylate
synthetase. It can also interfere with RNA synthesis. It has also immunosuppressant properties (Haskell, 1990; Dorr and Fritz, 1980).

2.2.1.3 Dose and dosage forms

**Intravenous**: 15 mg/kg body weight

**Topically**: 5-10% cream base

2.2.1.4 Pharmacokinetic parameters

**Absorption**

5-fluorouracil is commonly administered intravenously. Oral preparations such as tablets, syrups and solutions have been used, although in most of the cases absorption is unpredictable by this route. Responses after oral dosing appear to be shorter and fewer when compared with intravenous dosing (Hahn et al., 1975).

After oral doses of 5-fluorouracil 15 to 20 mg/kg/day, bioavailability may range from 50 to 80% (Jacobs et al., 1976). After a 200 mg oral dose of 5-fluorouracil peak serum concentrations may range from 0.5 to 1.0 Mg/l within 15 to 30 minutes, depending on the oral preparation used, and the presence or absence of food in the stomach.

**Distribution**

5-fluorouracil rapidly distributes to most tissues with a Vd of 8.84 ± 3.90 l/M². AUC is reported to be greater than 7125±237/μmole/l minutes following an intravenous dose of 500 mg/m² (Heggie, 1987). After a 15 mg/kg intravenous bolus dose, 5-fluorouracil penetrates the cerebrospinal fluid producing peak concentrations of 60 to 80 ng/l.

**Elimination**

Urinary excretion of intravenously injected 5-fluorouracil-2-14C, given as a single dose, amounts to only 11% in 24 hours; however, during this period, 63% of the radioactivity is expired. Given by continuous
intravenous infusion for 24 hours, plasma concentrations in the range of 0.5 to 3.0 μM are obtained and the urinary excretion of 5-fluorouracil is only 4% while the $^{14}$CO$_2$ excretion rises to 90% (Mukerjee and Heidelberg, 1960).

Pharmacokinetic parameters of the 5-fluorouracil could be summarized as follows (Table 2.3).

<table>
<thead>
<tr>
<th>Oral Absorption</th>
<th>28-100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Distributed into all body water by passive diffusion, crosses placenta, high and persistent levels in malignant effusions</td>
</tr>
<tr>
<td>$V_d$</td>
<td>0.25 l/kg, 8.84 l/m$^2$, 12-89% of body water</td>
</tr>
<tr>
<td>PPB</td>
<td>8-12%</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Metabolized in liver, most of the dose (80%) eliminated by liver</td>
</tr>
<tr>
<td>Excretion</td>
<td>60-80% excreted as respiratory CO$_2$, 2-3% by biliary system</td>
</tr>
<tr>
<td>Urine</td>
<td>15% as intact drug within 6 hours</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>10-20 minutes</td>
</tr>
<tr>
<td>CI</td>
<td>0.6-2.3 l/minutes, 16ml/ minutes/kg, women 155 l/hour/m$^2$, men 179 l/hour/m$^2$</td>
</tr>
</tbody>
</table>

**Metabolism**

5-flourouracil is metabolized extensively in the liver and its concentration declines rapidly to undetectable level within two hours. As plasma 5-flourouracil concentration declines, the concentration of its major metabolites, 5,6-dihydro-5-flourouracil (flourouracil-H2), $\alpha$-fluoro-$\beta$-ureido-propionic acid (FUPA) and $\alpha$-fluoro-$\beta$-guanido-propionic acid (FBAL) increases (Kirkwood et al., 1980). Fluorouracil-H$_2$ is detectable within 5-minutes of administration of 5-flourouracil with peak plasma concentration of 23.7 μmol/l occurring after one hour.
(Heggie et al., 1987). The liver converts fluorouracil\textsubscript{H\textsubscript{2}} to FUPA and FBAL by a dose-dependent saturable system. FUPA and FBAL peak serum concentrations are detectable approximately 90 minutes after infusion (Bruijn et al., 1986).

Fluorouracil is converted intracellularly to 5-fluoro-2-deoxyuridylate (FDUMP) by a series of enzymatic reactions. Initially, 5-monophosphate nucleotide (FUMP) is formed either by orotate phosphoribosyl transferase in the presence of 5-phosphoribosyl-1-pyrophosphate (PRPP), or by the action of uridine phosphorylase and then uridine kinase (Heidelberger, 1986, Figure 2.1). The FUMP is further metabolized to diphosphate (FUDP) and then to ribophosphate (FUTP), which can be incorporated into RNA thus producing a fraudulent RNA. However, the primary activation steps of fluorouracil involves the formation of the deoxymonophosphate (FDUMP) by the reduction with ribonucleotide reductase to FdUDP and then by the action of the phosphorylase to FdUMP.

Figure 2.1: Metabolism of 5-Fluorouracil

2.2.1.5 Interactions

Side effects due to 5-fluorouracil were increased when doxorubicin was given in combination with other drugs (Dorr and Von Hoff, 1994; Hansten and Horn, 1992; Tatro, 1992; McEvoy, 1994). These are recorded in Table 2.4.
Table 2.4: Interaction of 5-Fluorouracil with other agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopurinol</td>
<td>Decreased toxicity of fluorouracil</td>
<td>Possibly inhibition of thymidine phosphorylase, which activates fluorouracil</td>
<td>May decrease the hematological toxicity of fluorouracil, but results are conflicting</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Increased serum concentrations of fluorouracil</td>
<td>Appears to interfere with fluorouracil metabolism</td>
<td>Observe for increased toxicity of fluorouracil</td>
</tr>
<tr>
<td>Leucovorin</td>
<td>Increased cytotoxic and toxic effects of fluorouracil</td>
<td>Leucovorin stabilizes the bond to thymidylate synthetase</td>
<td>Some protocols are designed to take advantage of this effect; monitor toxicity closely</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Enhanced toxicity of fluorouracil</td>
<td>Decreased clearance of fluorouracil</td>
<td>Monitor for increased toxicity of fluorouracil</td>
</tr>
<tr>
<td>Thiazide diuretics</td>
<td>Increased myelosuppression</td>
<td>Decreased renal excretion of fluorouracil</td>
<td>Consider an alternative antihypertensive</td>
</tr>
</tbody>
</table>

2.2.1.6 Adverse effects

The toxic effects of 5-fluorouracil may be severe and some times may be fatal. The adverse effects are on the bone marrow and the gastrointestinal. Leucopenia, stomatitis, gastro-intestine ulceration and bleeding, or severe diarrhea, are the signs where treatment should be stopped (Dorr and Von Hoff, 1994; Schilsky, 1992; Krogh, 1993).

Cardiovascular

Severe chest pain, angina pectoris, fatal myocardial infarction and electrocardiographic changes in a patient receiving 5-fluorouracil by intravenous infusion were relieved following discontinuation.
Central Nervous System
Acute encephalopathy, acute cerebellar syndrome and manifestation of cerebellar dysfunction are major side effects. 5-fluorouracil also leads ‘organic brain syndrome’ with acute confusion, emotional liability, disorientation and impaired memory. Severe neurotoxicity in a patient given 5-fluorouracil, manifests as sluggish speech, cerebellar ataxia and confusion progressing to semi-coma.

Ocular
Excessive lacrimation, conjunctivitis and tear duct fibrosis are associated with the 5-fluorouracil.

Dermatologic
Alopecia, hyperpigmentation (over veins used), rash (extremities, sometimes on trunk), nail changes, photosensitivity, palmar-plantar erythrodysesthesia (hand-foot syndrome), radiation recall reaction (rare) and erythema, necrosis (topical application) are associated with the continuous administration of the 5-fluorouracil.

Hematologic
Myelosuppression, immunosuppression and megaloblastosis are major side effects of the 5-fluorouracil.