CHAPTER III

FLUOROURACIL

Fluorouracil is a fluorinated pyrimidine antagonist. Heidelberger et al. synthesized 5-FU in 1957. In this molecule, the hydrogen atom in position 5 of uracil is replaced by the similarly sized atom of fluorine, and the molecule was designed to occupy the active sites of enzyme target, thereby blocking metabolism in malignant cells. The drug occurs as a white to practically white to almost white, practically odorless, crystalline powder and is sparingly soluble in water, slightly soluble in alcohol and practically insoluble in chloroform and ether. The commercially available injection is colorless to faint yellow in color, the pH has been adjusted to approximately 8.6 – 9.4 with sodium hydroxide and hydrochloric acid if needed.[57-58] Structure of 5-FU is shown in fig. 2.

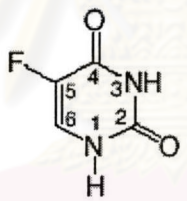

Figure 2 Structure of 5-Fluorouracil

Pharmacokinetics

Absorption [57-59]

5-FU absorption from gastro-intestinal tract after oral administration is unpredictable because of great variability of bioavailability. First pass metabolism in the liver as the main explanation for the low and variable 5-FU bioavailability. 5-FU is usually given intravenously. After IV administration, 5-FU is clear from plasma rapidly and no drug is detected after 3 hours. Little 5-FU is absorbed when applied to healthy skin.

Distribution[57-58]

5-FU is distributed into tumors, intestinal mucosa, bone marrow, liver and other tissues. Despite its limited lipid solubility, 5-FU readily crosses the blood-brain barrier and distributes into cerebrospinal fluid and brain tissue. Distribution studies in humans and animals have usually shown a higher concentration of the drug or its metabolites in the tumor than in surrounding tissue or in corresponding normal tissue. Within the target cell 5-FU is converted to 5-fluorouridine monophosphate and floxuridine mono-phosphate. It has also been shown that there is a longer persistence of fluorouracil in some tumors than in the normal tissues of the host, perhaps dues to impaired uracil catabolism. From these data, it has been suggested that the drug may possibly have some specificity against certain in comparison with normal tissues. 5-FU crosses the placenta in rats. It is not known whether the drug is distributed into human milk.

Metabolism

After penetration into the cell, 5-FU is metabolized via two routes in competition with each other: the anabolic route, which gives rise to the active metabolites, and the catabolic route, which inactivates 5-FU and leads to elimination of the drug from the organism.[60-61]

The Anabolic route

The anabolism of 5-FU is rather complex. (Figure 3) As itself, 5-FU is ineffective and needs to be anabolized to 5-fluorouridine-5'-triphosphate (5-FUTP), 5-fluorodeoxy uridine-5'-triphosphate (5-FdUTP), and 5-fluorodeoxyuridine-5'-monophosphate(5-FdUMP) to become effective as antimetabolite. 5-FU can react in the following three ways. The two ways both from 5-fluorouridine-5'-monophosphate (5-FUMP) via two stage phosphorylation by enzyme uridine phosphorylase and uridine kinase or in the direct conversion by orotate phosphoribosyl transferase. 5-FUMP can undergo two successive phosphorylations to give 5-fluorouridine-5'-diphosphate (5-FUDP) and then 5-FUTP, which

can be incorporated into RNA and an impairment of protein synthesis instead of uridine-5'-triphosphate (UTP). 5-FUTP can also be conjugated to sugar giving 5-FU–nucleotide sugars (5-FUDP-sugars).

The other way leads in two stages by thymidine phosphorylase and thymidine kinase to the formation of 5-FdUMP. 5-FdUMP inhibits synthesis of thymidine-5'-monophosphate (dTMP) by inhibition of thymidylate synthase. If combined with folinic acid (FA), the growth inhibitory effects of 5-FU are enhanced by stabilization of the ternary complex between thymidylate synthase, FdUMP and methylenetetrahydrofolate (Me-THF), one of the intracellular metabolites of FA, for a prolonged time period. Thus, the inhibition of synthesis of dTMP is enhanced and cell killing by impairment of DNA synthesis is increased.

5-FUDP and 5-FdUMP can be transformed into 5-fluoro-2'-deoxyuridine-5'-diphosphate (5FdUDP), which is then phosphorylated to 5-FdUTP. 5-FdUTP acts as a substance for DNA polymerases and can thus be incorporated into DNA. Fig. 3 shows anabolic route of 5-FU.

The Catabolic route

After administration of 5-FU, more than 80% of the injected dose is degraded according to the scheme shown in Figure 3. The first stage of this degradation occurs very rapidly under the action of dihydropyrimidine dehydrogenase (DPD), 5-FU is catabolised to 5,6-dihydro-5-fluorouracil (5-FUH_2). This stage effectively controls the rate at which 5-FU is available for anabolism. 5-FUH_2 is then degraded to give α-fluoro-β-ureidopropionic acid (FUPA). A third step leads to the formation of α-fluoro-β-alanine (FBAL), the major catabolite of 5-FU. Fig. 4 shows catabolic route of 5-FU.

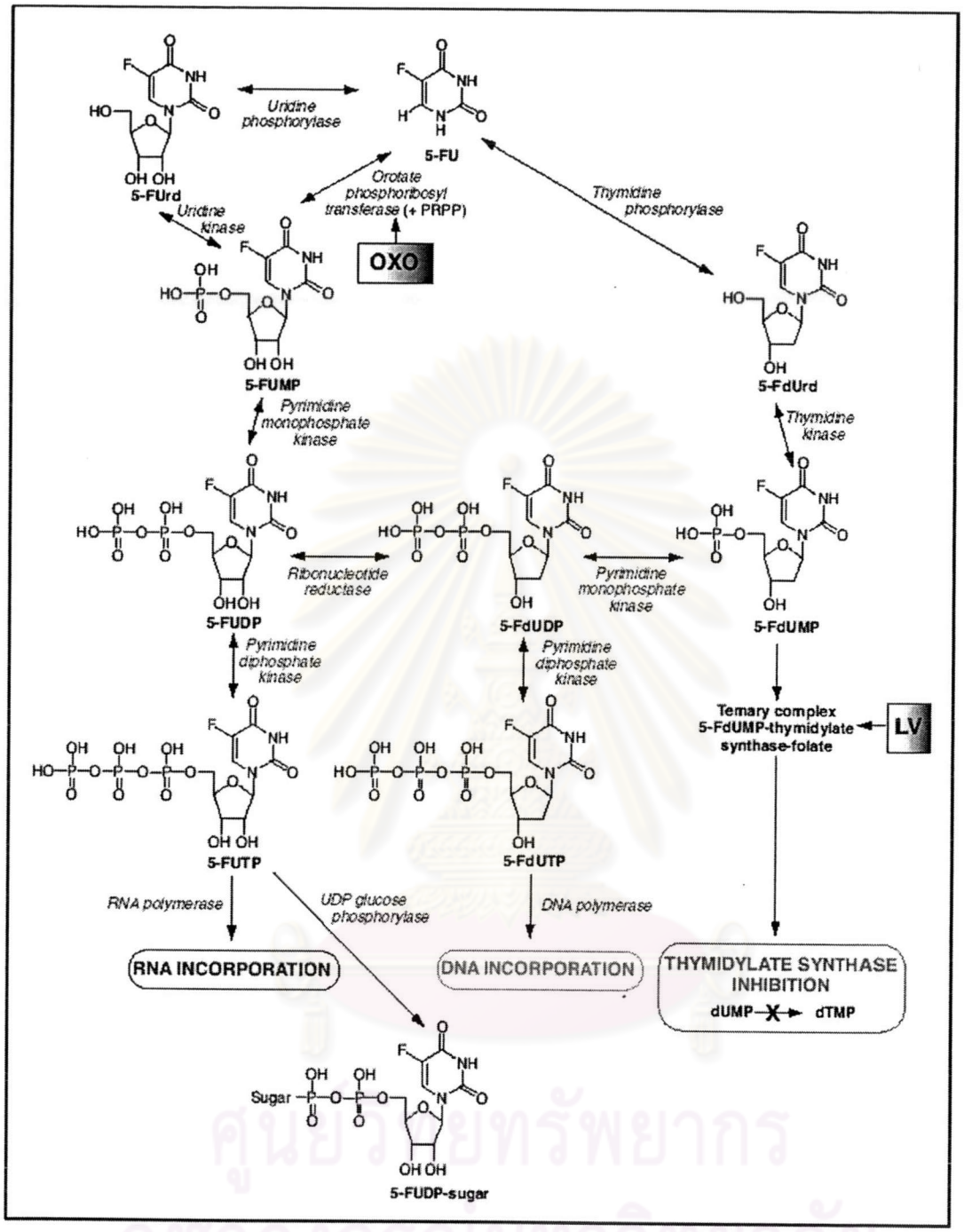

Figure 3 Intracellular anabolism of 5-Fluorouracil.

Abbreviations : 5-FUrd = 5-fluorouridine ; PRPP = 5'-phosphoribosyl-1-pyrophosphate ; 5-FUMP = 5'-flourouridine-5'-monophosphate ; 5-FUDP = 5'-fluouroruidine 5'- diphosphate ; 5-FUTP = 5'-fluorouridine-5'-triphosphate ; 5-FUDP-sugar = 5-FU-nucleotide sugars ; 5-FdUrd = 5-fluoro-2'-deoxyuridine ; 5-FdUMP = 5-fluoro-2'-deoxyuridine-5'-monophosphate ; 5-FdUDP = 5-fluoro-2'-deoxyuridine-5'-diphosphate ; 5-FdUTP =5-fluoro-2'-deoxyuridine-5'-triphosphate; dUMP = 2'-deoxyuridine-5'-monophosphate ; dTMP = thymidine-5'-monophosphate

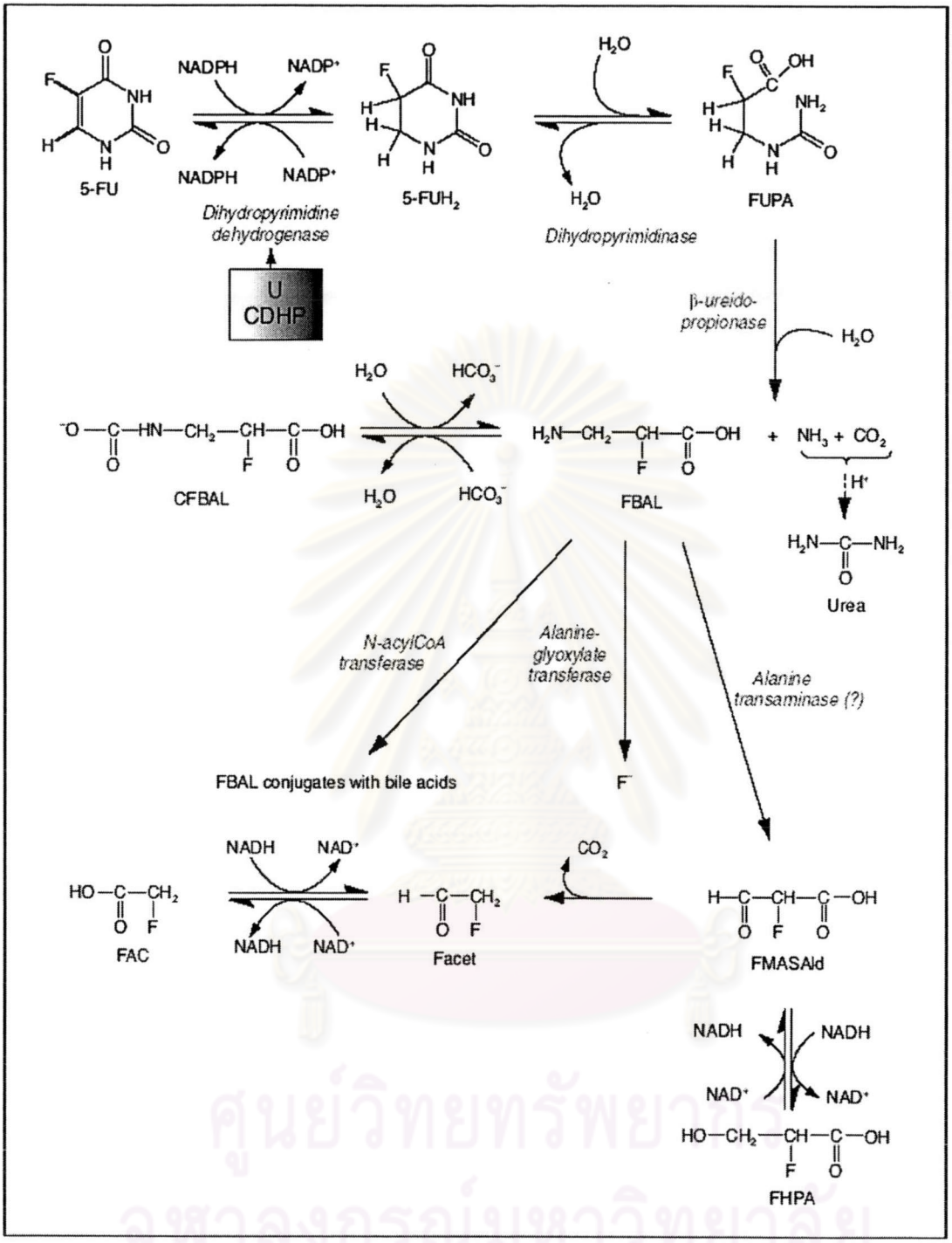

Figure 4 Catabolic pathway of 5-fluorouracil

Abbreviations : 5-FUH2 = 5,6-dihydro-5-fluorouracil ;

FUPA = α-fluoro-β-ureidopropionic acid ; FBAL = α-fluoro-β-alanine ; F⁻ = fluoride ion ;

FMASAid = fluoromalonic acid semi-aldehyde ; FHPA = 2-fluoro-3-hydropropionic acid ;

Facet = fluoroacetaldehyde ; FAC = fluoroacetate

Elimination

Following IV administration, 5-FU was rapidly changed intracellular to active metabolite. The quantities of 5-FU was rapidly clear from plasma. The plasma elimination half-life average about 16 minutes (range 8-20 minutes) and is dose dependent. Approximately 90% of a dosage of 5-FU is eliminated by metabolism (catabolism >anabolism), although less than 10% undergoes renal excertion. A small portion of 5-FU is anabolized in the tissue to 5-fluoro-2-deoxyuridine and then to 5-fluoro-2-deoxyuridine-5-monophosphate, the active metabolite of the drug. The rate-limiting step in the degradation of 5-FU involves reduction of the pyrimidine ring by dihydropyrimidine dehydrogenase (DPD). DPD concerts pyrimidines, such as uracil, 5-FU, to the dihydropyrimidine form. DPD is widely distributed in tissues throughout the body, including the liver, GI mucosa and peripheral blood mononuclear cell (PBMNC). Because the size of organ, the liver has the highest total content of DPD in the body and is a major site of 5-FU catabolism. The metabolites are excreted as respiratory carbon dioxide and as urea, alpha-fluoro-beta-alanine, alpha-fluoro-beta-guanidoproprionic acid in urine. Following a single IV dose of 5-FU, approximately 15% of an IV dose is excreted in urine within 6 hours; over 90% of this is excreted in the first hour. [57-58]

Correlation between DPD activity and 5-FU

In humans, the clearance of 5-FU is governed by the activity of DPD, an enzyme that indirectly influences the amount of 5-FU available to anabolism. DPD enzymatic activity is a labor-intensive assay that has generally limited its availability to research laboratories. The relationship between 5-FU and DPD activity is not tight in general study population, since the coefficient of correlation between DPD activity in lymphocytes and 5-FU plasma levels is only 0.34. Di Paolo et al. has reported only two from five patients with grade $\geq$ 3 toxicity were abnormally low DPD level while DPD level in the remaining three patients were superior to the cutoff value of 100 pmol/min/mg in partial DPD deficiency.[3,57]

Mechanism of action

From the metabolism of 5-FU gives rise to 5-FUTP. The atom of fluorine replacing the hydrogen on position 5 of uracil is of comparable size and during transcription, this fluoronucleotide thus mimics UTP and is recognized by RNA polymerases. This lead to the incorporation of 5-FU in all classes of RNA. It is thought that the full cytotoxicity stems from a combination of the numerous modifications of RNA due to incorporation of 5-FU rather than alteration of a single function.

The second way that 5-FU is activated, by the formation of 5-FdUMP, could be the most important. Indeed, this fluoronucleotide is an inhibitor of thymidtlate synthase (TS), which is involved in the synthesis of DNA. In the presence of the cofactor MeTHF serving as the methyl donor. TS and 2'-deoxyuridine-5'-monophosphate (dUMP) from a ternary complex, which enables transfer of a methyl group on carbon 5 of dUMP to form thymidine-5'-monophosphate (dTMP). Following 5-FU exposure and adequate 5-FdUMP formation, the methyl transfer does not take place because the fluorine atom in the C_5 position of 5-FdUMP is much more tightly bound than hydrogen. The enzyme is then trapped in a slowly reversible ternary complex. The formation of dTMP is therefore blocked, thereby decreasing the availability of thymidine-5'-triphosphate (dTIP) for DNA replication and repair. The genotoxic stress resulting from TS inhibition may activate programmed cell death pathways. [57-58, 62-63]

Limitations of the Activity of 5-FU

The activity of 5-FU is markedly limited by its rapid degradation into 5-FUH_2, via the action of enzyme DPD, the first enzyme in the catabolic cycles of 5-FU. It has been demonstrated that this enzyme deactivates more than 85% of the injected dose of 5-FU. Little drug is thus left for anabolism. Moreover, there is considerable variability in the activity of this enzyme between both normal and tumor tissues within one patient and among different patients.

There are also several other determinants of cellular sensitivity to 5-FU. Among these, the activity of enzymes involved in 5-FU anabolism, the level of TS activity or expression, the size of intracellular reduced folate and endogenous dUMP pools, the extent of 5-FUTP incorporation into RNA and 5-FdUTP into DNA, and the intratumor activity of DPD have been identified as important in determining response to 5-FU chemotherapy in patients.[64-65]

Effect of 5-FU

The toxic effects of 5-FU may be severe and sometimes fatal. The main adverse effects are on the bone marrow and the gastro-intestinal tract. Toxicity is schedule dependent: reducing the rate of injection to a slow infusion over several hours is associated with less hematological toxicity but may increase gastro-intestinal toxicity and hand-foot syndrome. Gastro-intestinal toxicity may also be exacerbated if 5-FU is given with FA.[57-58]

GI Effects

Anorexia and nausea are common adverse effects of 5-FU, and vomiting occurs frequently. These reactions generally occur during the first week of therapy, can often be alleviated by antiemetics, and generally subside within 2 or 3 days following therapy. Stomatitis is one of the most common and often the earliest sign of specific toxicity, appearing as early as the fourth day but more commonly on the fifith to eighth day of therapy. Diarrhea, which also occurs frequently, usually appears slightly later than stomatitis, but may occur concurrently or even in the absence of stomatitis. Esophagitis, proctilis, and GI ulceration and bleeding have been reported, and paralytic ileus occurred in two patients who received excessive dosage. Patients must be closely monitored for adverse GI effects. There is some evidence to suggest that the risk of GI toxicity may be increased in patients receiving 5-FU concomitantly with FA but additional study and experience are necessary to further elucidate the toxic potential of such therapy a GI syndrome

Hematological Effects

Leucopenia, predominantly of the granulocytopenic type, thrombocytopenia, and anemia occur commonly with 5-FU therapy; leucopenia usually occurs after an adequate course of 5-FU therapy. Pancytopenia and agranulocytosis also have occurred. The nadir of the white blood cell count usually occurs from the 9^{th} to the 14^{th} day after therapy is initiated but may occur as late as the 25^{th} day after the first dose of 5-FU, and counts usually return to normal after about 30 days. Maximum thrombocytopenia has been reported to occur from the 7^{th} to 17^{th} day of therapy. Hematologic recovery is usually rapid and by the 13^{th} day, blood cell counts have usually reached the normal range.

Dermatologic Effects

Hair loss occurs frequently and significant alopecia has also occurred. Regrowth of hair has been reported in patients receiving repeated courses of 5-FU. Partial loss of nail has occurred rarely and diffuse melanosis of the nails has been reported. The most common dermatologic toxicity is a pruritic maculopapular rash which usually appears on the extremities and less frequently on the trunk. This rash is generally reversible and usually responsive to symptomatic treatment. Dermatitis syndrome of erythema, pain and desquamation of the skin of plams and soles has been reported. Although particularly associated with administration by prolonged continuous infusions of high dose 5-FU, the rash may also occurred following bolus doses. These effects referred to as palmar-plantar erythrodysesthesia or hand foot syndrome, Symptoms generally response and may gradually disappear over 5-7 days after discontinuation of 5-FU, but adding oral pyridoxine has been reported to prevent or resolve these symptoms. Other dermatologic manifestation of 5-FU have included dry skin and fissuring, diffuse erythema, scalling and photosensitivity manifested by erythema or increased pigmentation. Exposure to strong sunlight may intensity skin reactions to the drug.

Nervous System Effects

Central neurotoxicity, including cerebellar ataxia, confusion, disorientation, and emotional lability is reported to occur rarely in patients receiving 5-FU. The incidence may be increased with high dose or intensive regimens. Patient with disorders of pyrimidine metabolism may be at increased risk of neurotoxicity.

Ocular Effects

Lacramination, dacryostenosis, visual changes, and photophobia have been reported in patient receiving 5-FU.

Cardiovascular Effects

Life-threatening cardiotoxicity (arrhythmias, ventricular tachycardia, and cardiac arrest, secondary to transmural ischemia) has been reported to occur in 0.55% of patients given 5-FU. The incidence in angina and less severe toxicity associated with coronary artery spasm may be higher. Pre-existing heart disease or mediastinal radiotherapy may be increase the risk as dose administration by prolonged infusion, but symptoms can be occurred by these risk factors. The exact mechanism is not known.

Other Adverse Effects

Rarely, anaphylaxis and generalized allergic reactions have occurred in patients receiving 5-FU. Fever that occurred during the end of the second week following the first dose of 5-FU, and which usually was not accompanied by demonstrable infection, has been reported. Epitaxis, thrombophleblitis, and vein pigmentation also have been reported.

Adverse effects of 5-FU is shown in table 2.

Table 2 Adverse effects of Fluorouracil[66]

ORGAN SITE	*SIDE EFFECT*	*ONSET*
cardiovascular	asymptomatic ECG changes (67%)	I
	angina pectoris (2-3%)	I
central nervous system	acute cerebellar syndrome (rare)	E and D
	acute encephalopathy (rare)	E and D
dermatologic	alopecia (mild)	E
	hyperpigmentation (over veins used)	E
	rash (extremities, sometimes on trunk)	E
	nail changes	E
	photosensitivity	E
	palmar-plantar erythrodysesthesia (hand-foot syndrome)	E
	radiation recall reaction (rare)	I
	erythema, necrosis (topical application)	I and E
gastrointestinal	mild nausea and vomiting	I
	stomatitis	E
	diarrhea	E
hematologic	*myelosuppression* nadir 7-14 days, recovery 22-24 days	E
	immunosuppression	E
	megaloblastosis	E
hypersensitivity	Type I (anaphylactoid, rare)	I
injection site	chemical phlebitis	I
ocular	excessive lacrimation	I
	conjunctivitis	I
	tear duct fibrosis	D

Dose-limiting side effects are underlined. I = immediate (onset in hours to days);

E = early (days to weeks); D = delayed (weeks to months); L = late (months to years)

Schedule Dependence

The activity and toxicity of 5-FU is depended on dosage and time of administration. Bolus injection of 5-FU inhibits DNA synthesis whereas continuous infusion may be inhibits of thymidylate synthase. Bolus injection is eliminated by saturated metabolism that is showed nonlinear relationship between dosage and plasma concentration. It is difficult to predict a change in blood concentration. In contrast, when 5-FU is administered by continuous infusion, plasma concentration is related with dose range. Accordingly, individual dose adaptation after continuous infusion for optimal 5-FU exposure is feasible.[7] By short-term infusion is prolonged time to expose 5-FU but elimination rate is similar to bolus injection, saturated metabolism may occurred.

It was known that bolus injection and short term infusion showed non-linear pharmacokinetic while continuous infusion showed linear pharmacokinetic. Pharmacokinetic parameters were also different among various dosage and duration of administration as shown in Table 3.

Table 3 5- FU pharmacokinetic parameters

Author	Regimen	Total body clearance	Half-life	Volume of distribution
Cano, et al.	IV bolus 5-FU 700-1000 mg total dose	0.39-1.47 L/min	-	-
	Continuous infusion 5-FU 700-1000mg (over 8 hr)	5.41-57.0 L/min	4-12 min	-
McMillian et al.	IV bolus 5-FU 11 mg/kg	0.55-2.67 L/min	11± 2 min	-
Heggie et al.	IV bolus 5-FU 500 mg/m^2	0.38-1.01 L/min/m^2	13 ± 7 min	9± 4 L/m^2
Bocci, et al.	IV bolus 5-FU 250 mg/m^2	54.64 ± 3.54 L/hr/m^2	0.17 ± 0.02 hr	9.14 ± 1.00 L/m^2
	IV bolus 5-FU 370 mg/m^2	25.43 ± 2.30 L/hr/m^2	0.21± 0.02 hr	4.15 ± 0.73 L/m^2
Larsson, et al.	IV bolus 5-FU 500 mg/m^2	1.203 L/min/m^2	-	18.046 L
	20 min infusion 5-FU 500 mg/m^2	1.851 L/min/m^2	-	same bolus injection
Fleming, et al.	Continuous infusion 5-FU 550-1000 mg/m^2/day	0.79-7.77 L/min/m^2	-	-

Monitoring AUC

Thyss et al. has described the significant relationship between area of the 5-FU plasma concentration-time curve and toxicity of chemotherapy in 29 non-metastases patients who received cisplatin in first day and followed of continuous intravenous infusion 5-FU for five days. They found a close relationship between 5-FU $AUC_{0\text{-}8\ hr}$ of patients more than 1800 mg/L.min and the prevalence in signs of toxicity. The limitation of this study was that patients received two chemotherapeutic agents. [67] Therefore it is not possible to identify the agent that caused adverse reactions. Later, Gamelin et al. [9] studied pharmacokinetics of 5-FU in colorectal cancer patients who received 8 hours continuous infusion in a dose of 1,000 mg/m^2 and increased 250 mg/m^2 every 3 weeks combined with fixed dose leucovorin of 400mg/m^2 to potentiate 5-FU efficacy. This study has shown that optimal therapeutic and nontoxic range of AUC and concentration at steady state (Css) are 960-1,440 mg./L.min and 2-3 mg/L^1, respectively. The risk of toxicity is related to $AUC_{0\text{-}8hr}$ more than 2,400 mg/L.min or Css more than 3 mg/L. Most studies showed pharmacokinetic data of 5-FU after continuous infusion but only a few studies provided pharmacokinetic parameters of 5-FU when given either in bolus or short term infusion. Larsson et al.[6] compared AUC of 20 min intravenous infusion and 2 min bolus injection of 5-FU 500 mg/m^2. Average AUC of bolus injection and short term infusion were 6158 ± 874 μmol/L.min (801.16 ±113.71 mg/L.min) and 3355 ± 428 μmol/L.min (436.48 ± 55.68 mg/L.min), respectively. AUC of 5-FU from different trial were shown in table. 4.

Table 4 5-FU AUC from varies regimen

Author	Regimen	AUC (mg/L.min)
Larsson et al.	500 mg/m^2 bolus 2 min weekly 500 mg/m^2 short infusion 20 min weekly	801.16±113.71 mg/L.min 436.48 ±55.68 mg/L.min
Gamelin et al.	1000 mg/m^2 continuous infusion 8 hr weekly increased 250 mg every 3 wk	960-1440 mg/L.min
Di Paolo et al.	370 mg/m^2 bolus 5 day every 4 week	555±37.8 mg/L.min
Grem et al.	425 mg/m^2 1 hr 5 day every 4 week	272.4± 130.2 mg/L.min

Matrix for concentration measurements

5-FU was detectable in whole blood, plasma and red blood cell. Whole blood is an ideal matrix for 5-FU concentration measuring because higher levels are attained, no sample processing required and small blood volume needed. However, 5-FU is not stable in whole blood. So it is recommended that blood sample for analysis should be placed on ice and determined 5-FU concentration as soon as possible. Red blood cell is used complicate process and requires largest blood volume in sample preparing. While plasma takes only one process and gave similar concentration result to whole blood and more stable until analysis. In this study, plasma is the matrix used to measure 5-FU concentration.[68]