CHAPTER II

REVIEW OF METHOTREXATE

Physico-chemical Properties (Reynolds, 1982)

Methotrexate (Figure 2) is a yellow to orange crystalline powder having a molecular weight of 454.4. It is practically insoluble in water, alcohol, chloroform, and ether, but freely soluble in dilute solutions of alkali hydroxides and carbonates, and soluble in dilute hydrochloric acid. Its pKa-values are 4.84 and 5.51 (Lippens, 1984).

Stability of Methotrexate in Aqueous Solution

To be dissolved in water, the drug is not completely stable. Thermal decomposition will occur at 85 °C and some N^{10}-methylpteroyl-glutamic acid is formed. Being exposed to ultraviolet light at room temperature (23 °C) will result in photodegradation of dilute methotrexate solution (0.1 mg/ml).

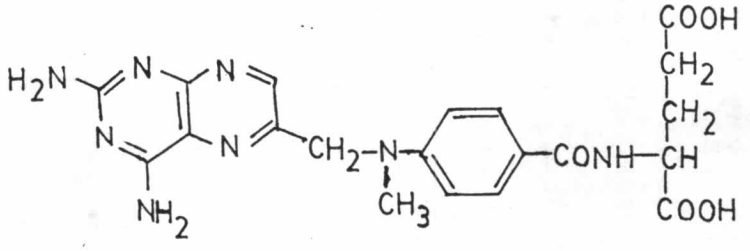

Figure 2. Chemical structure of methotrexate

The newly formed products are 2,4-diamino-6-pteridine-carbaldehyde, 2,4-diamino-6-pteridine-carboxylic acid and p-aminobenzoyl-glutamic acid. This photodegradation is accelerated by adding sodium bicarbonate to the solution. Photodegradation also occurs in room light, but to a lesser extent than in ultraviolet light. However, it is recommended that the methotrexate solution should be protected from the light if it takes more than 12 hours to infuse. The infusion solution should be prepared freshly before use (Chatterji, and Gallelli, 1978).

Methotrexate 2.5 mg/ml will be stable for 7 days at room temperature and at 30°C in sodium chloride injection, or lactated ringer's injection (Cradock, Kleinman, and Rahman, 1978).

Therapeutic Efficacy

Generally a variety of malignancies response to folate antagonists. Low doses are highly effective in choriocarcinoma, acute lymphocytic leukemia, breast carcinoma and intrathecal chemotherapy. But in high dosage, the drug is effective against malignant lymphoma, osteogenic sarcoma, epidermoid carcinoma of the head and neck, and small cell carcinoma of the lung (Bleyer, 1978).

Due to Frei and his co-workers' report, after first 24 hours of intravenous injection of 3 to 7.5 g/m^2 of methotrexate and every 6 hours during the following

72 hours the 10 mg/m^2 of leucovorin was given, the incidence of tumor regression was 50% in osteogenic sarcoma, and head and neck cancer, 59% in non-Hodgkin's lymphoma, 40% in small-cell lung cancer, 24% in prior chemotherapy breast cancer, and 50% in no prior chemotherpy breast cancer (Frei et al., 1980).

It also became an important therapeutic alternative in the treatment of severe psoriasis and in the suppression of graft-versus-host disease after bone-marrow transplantation, as well as in the experimental treatment of various rheumatic diseases after primary therapy had been failed (Jolivet, Cowan, Curt, Clendeninn, and Chabner, 1983).

Therapeutic Efficacy in Head and Neck Cancer

The management of head and neck cancer increasingly requires a team approach including surgical, radiation, and medical oncologists. In 1980, chemotherapy was generally employed for tumors that were not suitable for surgery and radiotherapy. In advanced state of disease, dramatic effects have been noted from the administration of chemotherapy prior to local intervention (Muggia, Rozencweig, and Louie, 1980). In 1982, a newer role of chemotherapy in this disease was in the initial treatment program. Pretreatment chemotherapy can debulk these lesions prior to surgery and/or irradiation in up to 80% of patients.

Post-treatment adjuvant chemotherapy still needed to be evaluated (Mead, and Jacobs, 1982)

1. Single Agent Chemotherapy.

Noting in conventional weekly doses (40 to 60 mg of methotrexate per square meter of body surface area, given intravenously), methotrexate is one of the most effective single agents in the treatment of recurrent head and neck cancers (Mead et al., 1982; Hong, and Bromer, 1983).

Woods and his co-workers randomized patients with advanced head and neck cancer to receive weekly intravenous methotrexate at doses of 50 mg per square meter, 500 mg per square meter, or 5 g per square meter. Patients who failed to response after 4 treatments at their initial doses were given 4 further treatments at the next higher doses. There were 2 complete responses and 21 partial responses to the initial dose - in 10 out of 22 patients given the high dose, 7 out of 27 given the medium dose, and 6 out of 23 given the low dose. A further 5 out of 16 patients responsed after crossing over to higher dose. There were an insignificantly improved response rate and duration of survival for the high-dose group. The toxicity was greatest in the patients who received the highest doses of methotrexate with 3 drug-related deaths (Woods, Fox, and Tattersal, 1981).

2. Combination Chemotherapy.

Cisplatin and methotrexate, the two most active agents for recurrent disease, have also been combined. This combination have potential for additive nephrotoxicity. Vogl and Kaplan combined moreover methotrexate (40 mg/m^2 on day 1 and 15), and cisplatin (50 mg/m^2 on day 4 every three weeks) with bleomycin (10 mg on days 1, 8, and 15). Thirty-seven patients were treated, with an overall response rate of 61%, and a complete response rate of 23%, and a median duration of 6 months. It appeared that toxicity was mild (Mead et al.,1982).

Although many clinical trials have investigated the effect of combination in recurrent head and neck squamous cancer there are few prospective randomized studies to compare combination chemotherapy with single-agent therapy. There is no evidence yet that multiple-drug regimens are superior to the standard single agents when response rate, remission duration, and overall survival are compared. The use of aggressive combination chemotherapy may cause serious toxicity and little therapeutic gain more than the best known single agents, methotrexate and cisplatin (Hong et al., 1983).

3. Combined-Modality Therapy.

The effectiveness of surgery or radiation therapy for small, locally confined tumors of the head

and neck has been well established, with 5-year survival rates of 70 to 80%. However, patients with either advanced primary tumors or regional nodal metastases have 5-year survival rates of 0 to 40% (Hong et al., 1983).

There are several theoretical mechanisms whereby combined therapy may improve cure rates. First, chemotherapy prior to definitive treatment, or "pretreatment" chemotherapy, may reduce the size of the tumor and facilitate surgery or render technically inoperable lesions operable. Second, a number of drugs may act as radiosensitizers and improve tumor response to irradiation. Third, chemotherapy given after definitive surgery/irradiation, or "post-treatment" adjuvant chemotherapy, may destroy micrometastasis (Mead et al., 1982).

Tarpley and associates treated 30 patients preoperatively with methotrexate (240 mg/m^2) and leucovorin rescue twice prior to surgery. In 77% of patients, there were some tumor shrinkages either at the primary or nodal disease site, but only 20% of patients had tumor shrinkages in both sites. When compared to a historical control group (surgical group), there was no increased incidence of postoperative complications and no difference in recurrence rate or survival. Many of the patients in this study had stages I and II diseases, and it might be difficult to improve upon their survival

with chemotherapy (Mead et al., 1982).

Methotrexate is an agent that produces S-phase block of the cell cycle causing an accumulation of cell in radiosensitive G1 phase (Berry, Hall, and Cavanagh, 1970). In a randomized study, Lustig and associates compared irradiation alone with methotrexate prior to definitive irradiation (25 mg intravenously every 3 days for a total of 5 doses) in 75 patients having stage III and IV squamous cell carcinomas of the head and neck. Distant metastases developed in 19% of the patients who received chemotherapy and irradiation when compared with 33% of the patients who received radiation therapy, but there was no difference in the 3-year survival. In this study, increased mucositis occurred in the combination-therapy arm (Lustig, Demare, and Kramer, 1976).

Adjuvant chemotherapy has been used in other solid tumors following definitive treatment to decrease the rate of distant metastases. Arlan treated 50 patients preoperatively with methotrexate and irradiation. Post-surgery, patients were treated with further irradiation and weekly methotrexate for a period of one year. The treatment was apparently well-tolerated, but long-term survival data were not available. Thus, there were no data to advocate routine use of post-treatment adjuvant chemotherapy outside the clinical trial setting (Arlan, 1976).

Mode of Action

Methotrexatehas been the most widely used as antifolate. Current concepts of the drug mechanism of action are illustrated in Figure 3. Methotrexate enters cell through the active transport system used by N^5-methyl-FH$_4$ and N^5-formyl-FH$_4$ (leucovorin and folinic acid), which is used as a rescue agent after high-dose-therapy (Jolivet et al., 1983). In addition to active transport, a second drug entry mechanism comes into play at high concentrations of methotrexate (in excess of 9 mcg/ml). This carrier-independent uptake provides a rationale for the clinical use of high-dose methotrexate (Hill, Bailey, White, and Goldman, 1979; Warren, Nichols, and Bender, 1978).

Reactions which are special importance in cellular proliferation are the biosynthesis of thymidylic acid, the nucleotide specific to DNA, and the biosynthesis of inosinic acid, the precursor of purines necessary for both DNA and RNA synthesis which were affected by methotrexate (Bleyer, 1978). After entering the cells, methotrexate will quickly bind to and inactivate dihydrofolate reductase. This enzyme has a crucial role in maintaining intracellular tetrahydrofolate (FH$_4$) by reducing dihydrofolic acid (FH$_4$), which is produced during thymidylate synthesis (Jolivet et al., 1983). Methotrexate appears to inhibit DNA synthesis to a greater extent than RNA synthesis,

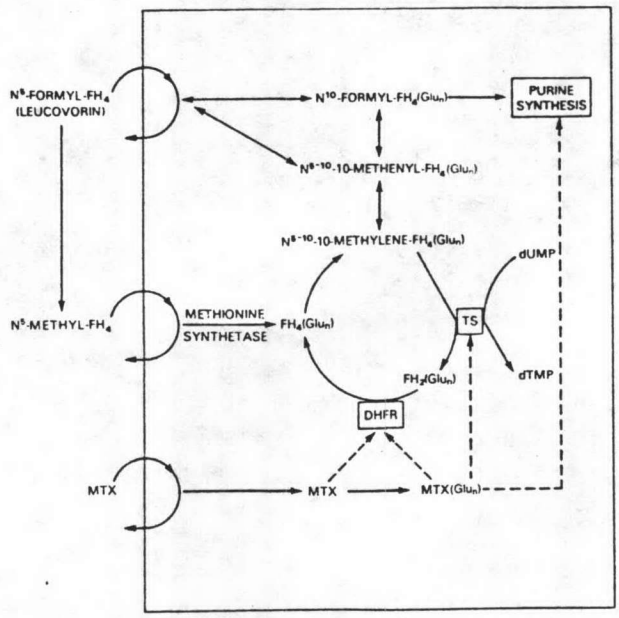

Figure 3. Mechanism of action of methotrexate. MTX denotes methotrexate, DHFR dihydrofolate reductase, TS thymidylate synthetase, FH_4 tetrahydrofolate, FH_2 dihydrofolate, Glu glutamyl, dTMP thymidylate, and dUMP dioxyuridylate. Broken line indicates enzyme inhibition.

suggesting that inhibition of thymidylate synthesis becomes the most important mechanism of methotrexate cytotoxicity (Hoffbrand, and Tripp, 1972).

Reduced folates (FH_4) are also required as co-factors in the conversion of glycine to serine and of homeocysteine to methionine. By preventing these amino acid interconversions, methotrexate may also interfere with protein synthesis (Bleyer, 1978).

The drug is highly cell-cycle dependent, acting primarily during DNA synthesis (S-phase). As a result, those tissues undergoing rapid cellular turnover with a high fraction of the cells in cycle are the most susceptible to the drug's cytocidal effects (Bleyer, 1978).

Citrovorum factor (5-formyl-FH_4) or leucovorin may be converted to 5-methyl-FH_4, which is the predominant reduced folate in the body. This metabolite is then converted to FH_4, and to N^{5-10}-10-methylene-FH_4 which will enter the reduced-folate cycle distal to the methotrexate-induced enzymatic block. Preliminary studies in patients with metastatic epitheliomas suggested that citrovorum factor was capable of effecting resumption of DNA synthesis in normal bone marrow without concomitant recovery of DNA synthesis in tumorous tissues (Frei, Jaffe, Tattersall, Pitman, and Parker, 1975).

Pharmacokinetic Studies

1. Animal Studies.

The pharmacokinetics of methotrexate had been studied in animals. Following administration of methotrexate 100 mg/kg of body weight in dogs, the maximum serum concentration was 147.2 mcg/ml. In serum, the distribution and elimination half-life were 12 and 90 minutes. The cumulative urinary excretion showed that the drug was rapidly eliminated, 31% of the drug were excreted by 8 hours, 34.9% by 24 hours, and 35.3% by 3 days (Porpaczy, Schmidbauer, Georgopoulos, and Endler, 1983).

Besides these Male Wistar rats weighting about 250 g received methotrexate 0.31, 3.1 and 31 mg/kg of body weight intravenously via the tail vein. For the highest dose (31 mg/kg), a triexponential description of the declining plasma concentration had to be used. For the 3.1 and 0.31 mg/kg doses, biexponential descriptions were sufficient to describe the data. The extrapolated initial concentrations C (C_{31} = 177 mcg/ml, $C_{3.1}$ = 9.65 mcg/ml and $C_{0.31}$ = 0.64 mcg/ml) were not proportion to the doses (Scheufler, 1982).

In rabbits, the intravenous kinetics of methotrexate (1.33, 4 and 12 mg/kg of body weight) could be described by a linear three-compartment model with a terminal half-life of 2.4, 2.7, and 3.6 hours,

respectively. During 8 hours, 50% of the dose were excreted unchanged form into the urine and 15% as the metabolite, 7-hydroxymethotrexate. These fractions remained constant with increasing dose. In continuous infusion experiments (9-900 mcg of methotrexate/kg X min) a decrease of the renal methotrexate clearance with increasing plasma concentration was observed (Iven, Brasch, and Engster, 1985).

2. Absorption.

In the dose of less than 30 mg/m^2 of body surface area, methotrexate is almost completely absorbed from the gastrointestinal tract. With doses of 80 mg of methotrexate/m^2 of body surface area or more, absorption from the gastrointestinal tract is protracted and incomplete, leading to plasma levels less than one-tenth that achieved after intravenous administration. These data implicate the presence of a saturable intestinal absorption mechanism (Henderson, Adamson, and Oliverio, 1965; Wan, Huffman, Azarnoff, Stephens, and Hoogstraten, 1974). Following intramuscular injection of methotrexate 15 mg/m^2 of body surface area, higher and more sustained concentrations occurs than that from oral administration of the same dose (Freeman-Narrod, Gerstley, Engstrom, and Bornstein, 1975).

3. Distribution.

After intravenous administration, methotrexate distributes rapidly within a volume of 18% of body weight and then within a space of 76% of body weight. These volume of distribution approximate to those of the extracellular space and total body water, respectively (Bleyer, 1978).

Patients with inoperable carcinomas of head and neck receive methotrexate by bolus intravenous injection at a dose of methotrexate 50 mg/m^2 of body surface area. Plasma concentration-time curve shows a biexponential decay when plotting semilogarithmically. The half-life of methotrexate in the undernourished group (40.43±9.48 hours) is significantly ($p<0.01$) prolonged, comparing to the better-nourished group (16.63±1.11 hours). In the undernourished group, the central compartment volume of 5.882±0.778 L showes a reduction, while the tissue compartment volume of 151.694 ±75.06 L indicates a defined increase, comparing to the better-nourished group with a central compartmant volume of 8.20±1.29 L and a tissue compartment volume of 86.99 ±11.517 L. On the contrary, clearance of 2.22±0.41 ml/min is markedly reduced in the undernourished group, comparing to a clearance of 4.18±0.29 ml/min in the better-nourished group (Rajeswari, Shetty, Gothoskar, Akolkar, and Gokhale, 1984).

Patients having advanced head and neck cancer, are treated with methotrexate 100 mg/m^2 of body surface area as an bolus intravenous injection. The plot of serum methotrexate decaying with time is fitted by a nonlinear regression analysis to a triexponential equation. The median total area under the concentration-time curve for methotrexate was 24.6 mcg/ml.hr (range 11.45 - 82.7 mcg/ml.hr). The median half-times of the three decay phases are 0.3, 3.09, and 33 hrs, but there are considerable interpatient variations. The median total plasma clearance was 115 ml/min and the median apparent volume of distribution at steady state is 37 L (Stewart, Margison, Wilkinson, and Lucas, 1985).

The approximate concentration ratios of plasma to tears and saliva are 21/1 and 333/1, respectively (Steele, Stuart, Whiting, Lawrence, Calman, McVie, and Baird, 1979).

Methotrexate is concentrated in the liver, with liver to plasma ratios of four at 3 hours, and eight at 24 hours after intravenous injection of methotrexate 80 mg/m^2 of body surface area. The amount excreted in bile may be inversely proportional to the dose that only 0.4% of the dose is detected in bile from one patient given methotrexate 80 mg/m^2 of body surface area intravenously (Bleyer, 1978).

Analysis at 37°C of fresh sera with 0.45 mcg/ml of methotrexate added and pH 7.4 from 19 healthy volunteers give a mean percentage bound 46.5±2.7% with range 41.5 - 51.0%. No significant reduction in the percentage bound is observed in increasing methotrexate concentration from $4.5X10^{-5}$ mcg/ml until it rises to 45.44 - 454.4 mcg/ml (Paxton, 1981).

4. Metabolism.

Methotrexateis metabolized in the liver by a mixed function oxidase, aldehyde-oxidase into 7-hydroxymethotrexate. Metabolism of methotrexate in man does not seem to occur to a significant degree. After high doses, however, 7-hydroxymethotrexate and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) are isolated and identified from the plasma and urine of patients, and methotrexate polyglutamates are detected in human liver (Balis, Holcenberg, and Bleyer, 1983).

5. Excretion.

With normal renal function, drug clearance from plasma approximates to 110 ml/min/m^2, more than 90% (>100 ml/min/m^2) of which are due to renal clearance. Nearly half of an intravenously administered dose is excreted unchanged form in the urine within 6 hours after administration, 90% within 24 hours, and 95% within 30 hours. With doses that produce plasma methotrexate concentrations up to 0.045 mcg/ml,

methotrexate renal clearance exceeds glomerular filtration rate by 1.5 to 2 fold, indicating that the antifolate is actively secreted by renal tubular cells (Balis et al., 1983).

Less than 2% of an intravenously administered dose is excreted in the stool as the parent compound and metabolites. Nearly all of the drug secreted in the bile are reabsorbed by the intestinal mucosa (Wan et al., 1974).

Toxicology

The principal toxicities with methotrexate are myelosuppression, gastrointestinal mucositis and hepatitis. Other toxicities observed depend on the type of regimen administered. Chronic low dosage administration is associated with cirrhosis, intestinal pneumonitis, osteoporosis, alopecia and immunosuppression, primarily T-lymphocyte dysfunction (Bleyer, 1978).

With high-dose systemic therapy, renal dysfunction, vomiting, acute desquamative dermatitis and B-lymphocyte dysfunction are also encountered (Bleyer, 1978). Neurotoxicity of intrathecal methotrexate therapy is also a major problem (Mott, Stevenson, and Wood, 1972).

Therapeutic drug monitoring of high dose methotrexate demonstrates an excellent correlation between the drug's toxic reactions and its pharmacokinetics. For each target tissue there are a critical minimal extracellular level (threshold concentration) and a critical minimal duration of exposure (threshold time), both of which must be exceeded for toxicity to occur. The severity of toxicity is directly proportional to the duration of methotrexate exposure beyond the time threshold, and relatively less dependent on the magnitude of methotrexate elevation above the extracellular concentration threshold (Balis et al., 1983).

For bone marrow and intestinal epithelium which were sensitive to methotrexate toxicity, the threshold concentration and threshold time appeare to be about 0.036 mcg/ml and 42 hours, respectively. Therapeutic drug monitoring has become indispensible for the management of patients receiving methotrexate therapy. Citrovorum factor will be continued until the plasma methotrexate concentration is less than 0.045 mcg/ml (Balis et al., 1983).