Therapeutic Drug Monitoring of Cytotoxic Drugs

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ABSTRACT
The majority of anticancer drugs are recognized with a narrow therapeutic index, the area under the plasma levels vs. time curve (AUC) is the common pharmacokinetic (PK) parameter, which utilizes specifically for cytotoxic drugs. Therapeutic drug monitoring (TDM) approach in these drugs has never been completely applied due to different reasons, for example, the use of combination chemotherapies for different malignant tumors, and the behavior of intracellular compounds; it is possible to eliminate these limitations by using specific concentrations of cytotoxic drugs and measure AUC after certain conditions. In this review article, we discussed the common TDM parameters, methods of analysis, and some of drug interactions for a group of cytotoxic drugs.

Keywords: Cytosine arabinoside, Cytotoxic drugs, Fluorouracil, Mercaptopurine, Methotrexate, Therapeutic drug monitoring (TDM).

RATIONALE FOR TDM

The TDM approaches can be carried out on cytotoxic medications if some of the prerequisites exist, the first one is the high dissimilarity in an inter-individual PK profile, by utilizing a clearance that expressed per m^2 (L/h/m^2) can comfortably detect this large variation, which is associated to the genetic profile of cancer patient, and the second prerequisite is the relationship among pharmacodynamics (PD) endpoints and the plasma concentration, as in the relationship between percentage counts decreasing of neutrophil vs. time, between pretreatment and nadir values. AUC represents the most applicable PK parameter for cytotoxic anticancer medications. PD endpoints are the best-correlated site in the cytotoxic AUC; this can appear in the maximum serum concentration after terminated of intravenous infusion. Another prerequisite is important to perform TDM approach on cytotoxic drugs will be in delay or postpone the measurement time of serum concentrations and PD endpoint [i.e., the AUC for short intravenous infusion (IV) infusion will be matched after 1 to 2 days with neutrophil and platelet nadir values, which detected in one or two weeks after chemotherapy course]. This elucidates the reason, which is behind the accurate timing of TDM applying in cytotoxic drugs (i.e., a specific AUC must

INTRODUCTION

Alkylating agents are used to treat malignant tumor by interfering with DNA bases by covalent bonds and therefore, inhibited DNA replication via making crosslinks in the double helix (examples: cyclophosphamide, busulfan, and carboplatin), anticancer antibiotics, which intercalate into DNA base pairs, and therefore, prevent replication by stopping reversible swiveling in the DNA replication fork (e.g., daunorubicin). Topoisomerase II will be an important target to be inhibited by the action of epipodophyllotoxins and result in separated DNA strands (e.g., etoposide, teniposide). Receptor binding is the mechanism of action of steroid hormones that ends with altering DNA synthesis (e.g., prednisolone). Mitotic spindle, which is responsible for segregating chromosomes to two daughter cells during mitosis, can be prevented by the action of vinca alkaloids (e.g., vincristine). Inhibition of purine and pyrimidine formation in order to stop DNA synthesis can be achieved by antimetabolites (e.g., methotrexate, 6-mercaptopurine).1 Cytotoxic medications provide a narrow therapeutic index, and there is insufficient pharmacological monitor of treatment, so they act as ideal agents to observe the development program of TDM.

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be determined at the end of drug administration). When the drug is already administered, there is no chance to make any modifications if the determined AUC was varying compared to the target AUC. Practically, the only condition that allows using a dose decreasing approach is unacceptable dose-limiting toxicity that noticed among cycles of therapy; because of this reason, TDM will supply detailed knowledge as guide to control dose regimen modifications in a therapy course. But this approach has some of disadvantages that limit its applications, as in chemotherapy, which is combined of drugs that require frequent plasma sampling to detect AUC values.

TDM AND PHARMACOGENETICS
The change in drug phenotype response is affected by different factors; one of them is the personal genetic profile that can vary the response via modulation of drug PKs and/or PDs profiles. Pharmacogenetics had been studied to investigate genes that are responsible for encoding drug transporters and metabolizing enzymes, this help to achieve effective drug treatment by decrease the possible personal adverse effects and increase the affinity of responders. Other factors that impact on PKs and/or PDs profiles can be pathophysiological status, drug-drug interactions, medication errors or allergies, diet, and environment.

CYTOTOXIC DRUGS
Leukemia protocol treatment is performed by utilizing groups of antimetabolite drugs as mercaptopurine (MP), methotrexate (MTX), and cytosine arabinoside (Ara-C), whereas treatment of solid tumors that can be presented in colorectal, breast, head, and neck cancers is carried out via fluorouracil (FU). Antimetabolite drugs mimic the natural substrates, such as, MTX is a folate analogue, FU and Ara-C are pyrimidines, and MP a purine. Therefore, they incorporated into DNA synthesis as a false base. The active intracellular metabolites of antimetabolites can be retained in a cell (Table 1). Controlling of dose regularly by the TDM approach is worked only with MTX.

MP
The MP is a prodrug, which is metabolized via three common routes: phosphoribosylation, oxidation, and S-methylation (Figure 1). The oxidative metabolic pathway, which is stimulated by xanthine oxidase will produce 6-thio analog of mercaptopurine (MP).

**Table 1:** Metabolic pathways and active compounds of antimetabolites

<table>
<thead>
<tr>
<th>Drug</th>
<th>Metabolic pathway</th>
<th>Active compounds</th>
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<tr>
<td>Methotrexate</td>
<td>Folate metabolism</td>
<td>MTX</td>
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<td></td>
<td></td>
<td>MTX polyglutamates</td>
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<td></td>
<td></td>
<td>7-OH MTX polyglutamates</td>
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<tr>
<td>Mercaptopurine</td>
<td>Purine metabolism</td>
<td>Nucleotide metabolites</td>
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<tr>
<td>Fluorouracil</td>
<td>Pyrimidine metabolism</td>
<td>Nucleotide metabolites</td>
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<tr>
<td>Cytosine arabinoside</td>
<td>Pyrimidine metabolism</td>
<td>Nucleotide metabolites</td>
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**Figure 1:** The intracellular of mercaptopurine: the principle metabolic routes are catalyzed by thiopurine methyltransferase (TPMT), xanthine oxidase (XO), and hypoxanthine phosphoribosyltranferase (HPRT)
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uric acid, and this enzymatic activity can be varied to 4 to 10 folds.

Hypoxanthine phosphoribosyltransferase (HPRT) is the enzyme, which is responsible for the first reaction in biological activation of mercaptopurine, this route ends with product: 6-thioguanine nucleotides (TGNs), which can achieve cytotoxic and immunosuppressive effect by certain mechanisms and also can inactive de novo purine synthesis. Thiopurine methyltransferase (TPMT) is the enzyme that stimulated S-methylation of the thiopurine.

Methods of Analysis
The high-performance liquid chromatography (HPLC) technology is used to quantify intracellular thionucleotide metabolites, TGNs, and methyl-MP nucleotides can be monitored by reverse-phase HPLC, UV radiation, and isocratic gradient elution. The most popular method is the thionucleotide hydrolysis, which is parent thiopurine before the HPLC quantification, by using gradient elution from an anion-exchange column, some of thionucleotide metabolites can be measured correctly. Also, in plasma it is possible to measure the major catabolite 6-thiouric acid. To measure TPMT metabolite by radiochemical or HPLC assays, it requires a sample of 100 µL packed RBCs or RBC lysate. Also, the genotype of TPMT can indicate its activity, i.e., some of the variant alleles are recognized for low activity of TPMT. The single nucleotide polymorphisms (SNPs) can regulate TPMT activity in an open reading frame; a study was carried out in 2000 by Yan L et al., to discuss the effect of thiopurine methyltransferase polymorphic tandem repeat and genotype-phenotype correlation analysis, they reached the result that different number tandem repeat within the TPMT promoter modulates the levels of RBC TPMT enzyme activity.

TDM
One of suggested chemotherapy of childhood acute lymphoblastic leukemia (ALL) is MP, serum level of MP can be utilized as a method of monitoring systemic exposure to MP. There is an alternative way of repeated blood sampling, via correlation of MP serum AUC with concentrations of MP in the urine. The correlations of PK-PD can measure RBC TGNs, so the drug metabolites in RBCs can be used as an indicator of therapy effectiveness in the treatment of ALL, especially with oral chemotherapy. In children with ALL who take the 75 mg.m⁻² of MP, the median TGN concentration was shown 275 pmol 8 x 10⁻⁸ RBCs. The relapse of this type of leukemia is correlated with lower TGN concentrations to be less 275 pmol and with the maximum activity of the pharmacogenetics enzyme TPMT. Old research in 1995 was performed by Schmeigelow K et al. to discuss the risk of relapse in childhood acute lymphoblastic leukemia, and its relationship to RBC methotrexate and mercaptopurine metabolites during maintenance chemotherapy; they found that any metabolite drug which depends on RBC TGNs and MTX polyglutamate concentrations will have a role in relapsing and failing of treatment. It is difficult to make a comparison among TGN concentrations per number of RBCs and the product of TGN and MTX concentrations per mmol of hemoglobin.

Monitoring Benefits
To achieve effective anticancer effect by MP chemotherapy, myelosuppression must be provided. In UK, the target of treatment of ALL is monitored myelosuppression, which is providing an alternative measurement of response toward thiopurine therapy in continuing chemotherapy. A study done in 1997 by Lennard L. et al., to know the influence of inherited thiopurine methyltransferase activity on drug metabolism and cytotoxicity, when used thiopurine drugs in the treatment of childhood leukemia, concluded that there is a negative correlation between active TPMT in the diagnosis of the disease and the duration of cytopenia, which driven MP withdrawal continuing therapy. Unfortunately, any patient with high TPMT activities will fail treated by a standard dose of MP; also, they do not show any cytotoxic activity. The possible cause of 6MP cytotoxicity is the presence of heterozygous phenotype TPMT activity.

Drug Interactions
Thiopurine cytotoxicity can occur in case of inhibition of MP oxidation or S-methylation metabolism by another drug which taken at same time with MP, allopurinol, the standard aspirin dose, and olsalazine are inhibitors of xanthine oxidase enzyme, and it is recommended to decrease the allopurinol dose by two-thirds, if it is taken concurrently with 6MP prodrug azathioprine. The diuretic agent: furosemide can interfere with S-methylation of thiopurines, as furosemide inhibits TPMT with an IC₅₀ of 170 µM, and TPMT may influence with disulfiram therapy for alcoholism.

MTX
The MTX is a competitive inhibitor of dihydrofolate reductase (DHFR) enzyme that is responsible for transforming reaction of folates into tetrahydrofolate. The reduced form of folate transporter is important to carry the carbon atoms, which are required in de novo purine synthesis and for methylation of uracil into thymine in DNA synthesis. Leucovorin is a folate analog used as cell destructive prophylaxis from MTX inhibition. The mechanism of action of leucovorin is illustrated in Figure 2. The toxicity can be monitoring via polyglutamation.

Methods of Analysis
Commercial kits are used to measure MTX levels in biological fluids in plasma, serum, urine, and cerebrospinal fluid (CSF). The basic work of these kits depends on radioimmune, radioenzymic, and enzyme fluorescence polarization immunoassay. Fluorescence polarization (TDx) and enzyme immunoassays (EMIT) are using sample volume equal to 50 mL serum, and this is considered a small volume, whereas radioenzymic and radioimmune assays are using greater volumes equal to 300 mL plasma. HPLC can be used to determine the quantity of MTX metabolites.
TDM

The MTX works as a cytotoxic agent to remove malignant cells; it can cause different dangerous adverse reactions, for example, myelosuppression, gastrointestinal mucositis, and hepatic cirrhosis. The main target, when started treatment with MTX is to maintain its elevated plasma concentration ($10^{-4} - 10^{-5}$ M) for a longer period of time (approximately 12 to 36 hours). In the treatment protocol of childhood ALL, MTX is given at five cycles in high dose in the first year; this is started with 500 mg.m$^{-2}$ over 24 hours, with leucin rescue coovat 48 hours, in accordance with initiating of infusion. MTX clearance in the first cycle is based on its serum level at 1 and 6 hours after starting the infusion, also with monitoring the dose at 8 hours to maintain the aimed exposure, which is 580 to 950 mM MTX, this happens for the rest of cycles.

Drug Interactions

The chemotherapy for childhood ALL includes using MP and MTX concurrently; MTX can inhibit folate compounds, which involved in de novo purine synthesis as xanthine oxidase enzyme; this will increase competition on thiopurine MP, and end up to produce intracellular thionucleotide metabolites.

FU

Fluorodeoxyuridine monophosphate (FdUMP), is the metabolite of fluorouracil that work on thymidylate synthesis inhibition, FU is metabolized in the liver by the effect of dihydropyrimidine dehydrogenase (DPD) enzyme, which is responsible for rate-limiting step and for degradation more than 80% of administered FU, its activity can be evaluated in the peripheral blood mononuclear cells (PBMC). Figure 3 represents FU metabolism.

Methods of Analysis

Using reverse-phase HPLC with UV detection is facilitating quantification of FU plasma concentrations; dihydropyrimidine dehydrogenase (DPD) is regulator for FU in tissues. A study performed in 1993 by a team of researchers to discuss the activity of dihydropyrimidine dehydrogenase in human peripheral blood mononuclear cells and liver and its relation with clinical implications in 5-fluorouracil chemotherapy, they isolated mononuclear cells by standard techniques, and they stored them -70$^\circ$C in a fetal calf serum to be used later.

Familial deficiency in dihydropyrimidine dehydrogenase recorder elevated in plasma uracil concentrations, so specialist physicians can use uracil serum level as a clinical marker to diagnose the deficiency of DPD.

TDM

Relationship between fluorouracil systemic exposure and tumor response and patient survival was discussed in 1994 by a group of scientists; they found a correlation among FU plasma level and the response rate and patient survival. Cytotoxicity can be easily predicted by FU area under the serum concentration curve (AUC); also, the FU doses could be adjusted by nomograms according to plasma concentration. There is a relationship between low PBMC-DPD activity and liver DPD activity. Head and neck cancer patients have a response to FU therapy, and very low DPD activities in tumor compare to non-treated with FU. Therefore, DPD activities can be used as an indicator of the response of FU in cancer patients.

Drug Interactions

Therapeutic effect of FU can be improved by inhibition of DPD activity; 5-ethynyluracil is a potent DPD inhibitor that can be administered concurrently with the standard dose of FU. Antiviral agent sorivudine can stimulate FU toxicity in Japanese patients after few days of the administration, because of bromovinyluracil, which is a potent metabolite of sorivudine that inhibits DPD.
ARA-C

Phosphorylation is controlling the Ara-C cytotoxicity, this occurred via cytidine kinase to cytosine arabinoside triphosphate (Ara-CTP), which is the active therapeutic form, and it has a role in DNA polymerases inhibition (Figure 4).

Cytidine deaminase is the enzyme responsible for inactive Ara-C into uridine arabinoside (Ara-U). So the phenotype of Ara-C deamination can be determined via plasma Ara-U/Ara-C ratio.

Methods of Analysis

Ara-C and Ara-CTP levels have been determined in leukemic blasts and normal mononuclear blood cells. Measurement of plasma Ara-C and Ara-U with HPLC has very limited detection, approximately, 10 ng.mL⁻¹ Ara-C, and the levels of Ara-CTP in leukemic blast cell was detected in lower limit of 5 pmol on the column. To detect H³ Ara-C and other metabolites (Ara-U, Ara-UTP, and Ara-CTP), ion-pair HPLC with solid-phase scintillation techniques are used. The range detection is 40 to 200 pg in a column. The only metabolite which may be determined in vivo is Ara-CTP, which is also PKs can optimize Ara-C treatment.

TDM

Ara-C is administered parenterally to achieve the preferred absorption; if it is given orally, this will lose its absorption by directly converting to inactive form Ara-U by the intestinal mucosa. In a comparison done in 1999 with two samples of bone marrow biopsies to measure Ara-C metabolism, one sample is taken from patients with acute myeloid leukemia (AML), and the second was a standard (healthy state). The result shows no differences in the production rate of the deamination product Ara-U.

Specific Concerns in Pediatric Patient Populations

Practically, it is possible to accept body surface area-based dosing in pediatric patients with cancer, this is because of the correlation among body size and drug elimination, and the concerns of cytotoxic medications, which have narrow therapeutic index, and dosing inconsistencies are common in young children, especially with treatment protocol based on body weight. Children who are treated below a limited weight or age, dosing reduction approach should be performed for them.

Limitation of TDM for Cancer Chemotherapy

There are many factors, which restrict applying TDM approach:

- There exists an imperfect understanding of the pharmacology and PKs of most antineoplastic agents.
- The plasma drug concentration is an indirect measure of the amount of drug in the target tissue.
- Difficulty in identifying concentration-effect relationships because antineoplastic agents are almost always given in combination.

Figure 3: Fluorouracil (FU) metabolic steps: FU can either be catalyzed by dihydropyrimidine dehydrogenase (DPD) to form dihydro FU or phosphorylated to form active nucleotide metabolites; The nucleotide metabolites depicted above are fluorouridine monophosphate, diphosphate, fluoro-deoxyuridine diphosphate, monophosphate (FUMP, FUDP, and FdUMP, respectively); Intracellular metabolism of FU and its nucleotide is analogous to uracil metabolism; FU is converted to FUMP via 5'-fluorouridine in a two steps reaction catalyzed by nucleoside phosphorylase and uridine kinase; It is responsible that FUMP could be formed directly from the FU base via pyrimidine phosphoribosyltransferase; Phosphorylation of FUMP proceeds by nucleoside monophosphate kinase; nucleoside 5'-diphosphate reductase (ribonucleotide reductase) catalyzes the production of 2'-deoxyribonucleotide (FdUDP) from the ribonucleotide (FUMP); phosphate is removed from nucleotides by 5'-nucleotidases.
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There is a naturally long lag time between the measurement of plasma concentration and the definitive pharmacodynamic effect.

Cancer is a group of heterogeneous diseases, which inherent characteristics that affect the concentration-effect relationship for antineoplastic agents.

CONCLUSION

Secondary tumor, which is associated with alkylating agents and epipodophylotoxins can occur as a result of failed primary treatment. This stimulates late cardiac toxicity endowed by the anthracyclines. In order to develop PK models and randomized studies need more efforts to estimate the TDM approaches in cytotoxic drugs. The oncological community requires more encouragement to achieve trustful studies and to promote whole treatment techniques.

REFERENCES


Figure 4: Cytosine arabinoside (Ara-C) metabolism: determination to uracil arabinoside (Ara-U) and the formation of the intracellular nucleotides cytosine arabinoside mono-di and tri-phosphate and arabinofurosyl-uridine monophosphate (Ara-CMP, Ara-CDP, Ara-CTP, and Ara-UMP, respectively) analogous of the endogenous deoxynucleotides; the deamination of Ara-C catalyzed by (deoxy) cytidine deaminase and Ara-CMP by dCMP deaminase; the formation of Ara-CMP is catalyzed by (deoxy) cytidine kinase; Subsequent phosphorylation of the monophosphate proceeds by nucleotide monophosphate kinase (1) and nucleoside diphosphate kinase (2).


