[14:50 - 15:30]

Significance of genetic polymorphism on appropriate use of drugs

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There is great variation in the way individual response to medications, with regard to both host toxicity and treatment efficacy. The effects of drug are well known to be dependent on the plasma concentration of the active form of drug, not on drug dose. Therefore, in the case of drug of which the occurrence of efficacy or side effect is related to the plasma concentration, in general, TDM (Therapeutic Drug Monitoring) is clinically conducted to maintain of drug concentration within therapeutic concentration range. On the other hand, potential causes for variability in drug effects include the pathogenesis and severity of the disease being treated and concomitant disease; the individual's age, renal and liver function and nutiritional status; and drug interaction. Although there are several factors which cause individual difference in the effects of drugs clinically used, it is now recognized that individual differences in the metabolism and disposition of drugs can have a greater influence on the efficacy and toxicity of drugs. The significance of individual difference in the activity of drug-metabolizing enzyme depends on clinical importance for the activation or inactivation of drugs. Individual differences in pharmacokinetics of drugs may be associated with toxicity of drugs that have a narrow therapeutic window and are inactivated by drug-metabolizing enymes. In contrast, in case of the drugs that are activated by metabolic process, efficacy of medications may be reduced. The main causes for the individual difference in drug metabolism are also: (1) induction or inhibition due to concomitant drug therapies, nutrients or environmetal factors; (2) genetic polymorphism; (3) physiological status; and (4) disease states.

Table 1 Individual difference in drug metabolism
Induction or inhibition due to concomitant drug therapies
Induction or inhibition due to environmental factors
Genetic polymorphism
Physiological and nutritional status
Disease states

The drug interaction in concomitant drug therapies and genetic polymorphism appear to be major important factors for the occurrence of adverse effects or the quantitative or qualitative change of therapeutic efficacy.

Polymorphism of human P450 enzymes

The alleles that cause defective, qualitatively altered, diminished or enhanced rates of drug metabolism have been identified for many of the P450 enzmes. Major molecular mechanisms for alteration of P450 enzyme activities are shown in Table 2.

Table 2 The major mechanisms of alteration of P450 activities
Splice site mutations resulting in exon skipping
Gene duplication or multiduplication
Point mutations resulting in early stop codons
Point mutations resulting in amino acid substitutions that alter protein stability or catalytic activity
Complete gene deletions

Completely inactivated alleles have been found for P450 enzymes such as *CYP2D6, CYP2C19 and CYP2A6.* Defective alleles can be the result of gene deletions, gene conversions with related pseudogenes and single base mutations causing frameshift, missense, nonsense or splice-site mutations. Individuals having such defective alleles will be predisposed to drug toxicity or inefficacious medication due to an impaired ability to metabolize drugs. For example, since patients possessing CYP2C9 variant

show low clearance of (S)-warfarin, they may be fully anticoagulated with a dose less than standard dose of warfarin. CYP2C9 is also important in clearance of phenytoin and tolbutamide, both potentially very toxic drug in excess. In contrast, losartan which is prodrug, will be poorly activated and inefficacious with CYP2C9 deficiency.

The genotyping of patients undergoing peripheral blood stem cell transplantation

Busulfan, an alkylating agent, widely used for many high-dose conditioning regimens before bone marrow transplantation, and is one of the few agents to replace total body irradiation. Since low exposure to busulfan has been associated with an increased risk of graft rejection, especially in the setting of mismatched transplantation, high-dose regimen is necessary to obtain successful engraftment although a high dose of busulfan increases the incidence of busulfan-related toxicities. Therefore, to avoid convulsions, one of the toxicicity due to high-dose pre-medication with busulfan, phenytoin is administered during busulfan treatment as standard regimen. Since phenytoin have a narrow therapeutic window, TDM is generally conducted to maintain the concentration of phenytoin within therapeutic range or to confirm whether the dose is appropriate. In the course of the TDM operation, we have experienced some cases in which genotyping of CYP2C9 responsible for metabolism of phenytoin, was also needed and useful tool in addition to TDM for confirmation of the mechanism of abnormal pharmacokinetics of phenytoin. In one case, phenytoin dose was reduced according to the results obtained from TDM, but the control of concentrarion of phenytoin within therapeutic range could not be succeeded and adverse reaction which is considered to be due to phenytoin over dose was also continued. This case showed that TDM is not necessarily enough to prevent the occurrence of adverse reaction of phenytoin. In another case, the patient who underwent peripheral bood stem cell transplantation to cure acute lymphoblastic leukemia about 2 months ago, experienced convulsions which appeared to be induced by tacrolimus. Tacrolimus was replaced by cyclosporin, and phenytoin was administered to prevent convulsions. Since the trough

concentration observed to be higher than the upper limit of therapeutic range, phenytoin treatment was discontinued. A high serum phenytoin concentration suggesting that the patient had a poor metabolic rate, possibly through possession of the *CYP2C9*3* allele. However, no blood samples before peripheral bood stem cell transplantion remained in this case. Therefore, after obtaining consent, urine and current blood was collected.

In this case, the *CYP2C19* genotype obtained from the urine (*CYP2C19*1/*1*) was clearly different from that of the blood (*CYP2C19*1/*2*), indicating that urine is a useful source of genomic DNA derived from the patient's somatic cells. Unexpectedly, results obtained from the urine indicated that the patient possessed a homozygous wild-type of the *CYP2C9* gene (Figure 1), strongly suggesting that the abnormal pharmacokinetics of phenytoin were not due to genetic polymorphism of CYP2C9 or CYP2C19. In this case, genotyping accelerated the discovery of a drug-drug interaction, and enabled exclusion of hereditary factors as a possible reason for unusual drug pharmacokinetics. Although the precise sourse of genomic DNA in the urine is unknown, it may be cell debris derived from renal collecting tubules and/or bladder. It should be mentioned that genotyping with urine may not succeeded if the patient pyuria, since the recipient DNA may be mixed with blood DNA produced by transplanted stem cells. However, urine may be the best sample from which to obtain orignal genomic DNA from patients who have undergone peripheral bood stem cell transplantation or bone marrow/cord blood transplantation.

At present two major techniques are available. One is widely used in clinical practice in which to adjust treatment dose, the bood concentrations of drugs are measured after drug medication was started. Another is to analyze the presence of polymorphism before drug treatment. Both phenotyping and genotyping are important techniques for evaluating of individual drug-metabolizing capacities and appropriate use of drugs.