Thursday 21 November

PLENARY SESSION 5

Pharmacogenetics

210

General overview of pharmacogenetics

M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institut fuer Klinische Pharmakologie, Stuttgart, Germany*

There are in principle 3 mechanisms by which genetic polymorphisms or rare mutations of proteins involved in the metabolism, transport and action of drugs can lead to heterogeneity in efficacy and toxicity of drugs.
Drug metabolizing enzymes, in particular cytochrome P450 enzymes play a pivotal role in the elimination process of most drugs. Variability of drug metabolism is responsible for the prononounced interindividual differences in plasma concentrations when patientd receive the same dose of a drug. As a consequence variability in drug action and side effects / toxicity ensues. During the last 30 years for many phase 1 and phase 2 enzymes catalysing the biotransformation of drugs mutations have been identified. These mutations affect either the expression or catalytic properties of these enzymes. In the case of mutations leading to a loss of function administration of a standard dose of a drug will lead to very high plasma concentrations resulting in exaggerated response, side effects or toxicity. On the other hand gene amplification of enzymes resulting in ultrarapid metabolism of drugs has been identified as a mechanism of poor response. Moreover, in the case of prodrugs which require bioactivation for therapeutic efficacy loss of enzyme function due to polymorphism of the enzyme is associated with a loss in efficacy.
But even if drug dose is individualized guided by therapeutic drug monitoring in order to achieve the same plasma concentrations substantially variability in therapeutic response and side effects will still be observed because concentrations at the site of action vary substantially. It is increasingly recognized that transfer of drugs in and out of the cells is not a passive process depending on physicochemical properties, lipophilicity and protein binding but also involves active transfer by transport proteins. These transport proteins are expressed in many tissues and affect the absorption, biliary and renal excretion of drugs. They constitute the blood brain barrier (BBB) for many drugs since they are expressed at the luminal site of the endothelial cells of the brain capillaries limiting the transfer of drugs from the blood into the CNS. For a number of these transporters genetic polymorphisms have been discovered which affect their expression. Finally, same concentration of a drug at the site of action does not necessarily mean identical response because mutations at drug targets (receptors, neurotransmitter transporters, signaling pathways) can profoundly alter response.

211

Pharmacogenetic variation of UDP-glucuronosyltransferases in drug resistance and cancer

Brian Burchell, Brian Ethell, Jeff Cummings. *Ninewells Hospital & Medical School, Department of Molecular and Cellular Pathology, Dundee, UK*

Extensive inter-individual and inter-ethnic differences in drug glucuronidation have been reported. These variations in drug glucuronidation are caused by genetic polymorphisms and differential expression of UDP-glucuronosyltransferases (UGTs) induced by environmental chemicals.
Sixteen functional human UDP-glucuronosyltransferases (UGTs) have been identified and classified into two subfamilies based on sequence similarity. All of the UGTs are able to catalyse drug glucuronidation in vitro.
Genetic defects have been observed in the UGT-1A1 gene associated with hyperbilirubinaemia. In familial Gilbert's disease, mild hyperbilirubinaemia is caused by reduction in promoter function and expression of UGT1A1. Hyperbilirubinaemia caused by various drugs has been shown to be associated with this frequent polymorphism in the UGT1A1 gene.
Mutations and polymorphisms have also been examined in several other UGTs and linked to variation in xenobiotic glucuronidation and cancer.
Resistance mechanisms of human colon cancer to cytotoxic drug chemotherapy are poorly characterised. Two human colon cancer cell lines and two different cytotoxic agents (SN-38 the active metabolite of clinically used CPT-11 and NU/ICRF 505 an anthraquinone-tyrosine conjugate) have been used to identify a novel drug resistance mechanism caused by drug glucuronidation. The presence of UGT1A9 enzyme and activity was detected in drug resistant HT29 cells, but not in sensitive HCT116 cells. Propofol, a probe substrate for UGT1A9, competitively inhibited glucuronidation of SN-38 and NU/ICRF 505 by over 80% resulting in up to a 32 fold elevation in intracellular drug concentration. Co-treatment of cells with propofol and NU/ICRF 505 or SN-38 increased cytotoxicity by 5 and 2 fold respectively in HT29 cells. A range of UGT aglycones including food additives (methyl 4-hydroxybenzoate) and proprietary medicines (ibuprofen) were capable of inhibiting SN-38 and NU/ICRF 505 metabolism. Glucuronidation may represent a major mechanism of intrinsic drug resistance in colon cancer amenable to reversal with relatively non-toxic agents.
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212

Pharmacogenetic determinants of clinical outcome and toxicity in colon cancer

H. Lenz, *Keck School of Medicine, USC, Los Angeles, USA*

We have identified predictive markers (gene expression and genomic polymorphisms) of response and survival to both fluoropyrimidines and platinum based chemotherapeutics in colon cancer. These markers include TS, DPD and TP as predictive markers for fluoropyrimidines chemotherapy and ERCC1, XPD, and GSTP1 for platinum based therapy.TS expression has been correlated with disease free survival, overall survival and is predictive of recurrence (independent of patients who receive 5-FU).The predictive value of TS has also been demonstrated to identify those patients whose tumors may respond to fluoropyrimidines, as those with low levels of TS are more likely to respond. Other markers contributing to determining the response to fluoropyrimidines based therapy include thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD). Using these markers in combination increases the likelihood in predicting whether a patient will responds to therapy. Interesting a genomic polymorphism in the 5' UTR region of TS also predicted response to 5-FU based chemotherapy as well as toxicity to protracted infusion of 5-FU and Xeloda.Recently, newer platinum drugs specifically oxaliplatin have shown significant activity in colorectal cancer. The expression levels of genes found to be vital in platinum metabolism have revealed potential markers to predict responses. With the use of intratumoral gene expression of ERCC1 and genomic polymorphisms of ERCC-1, XPD and GSTP-1, we were able to predict response and survival in patients with colon cancer treated 5-FU/oxaliplatin. The capability of an almost absolute prediction of non-response as well as identification of a set of patients with very high but not absolute probability of response has a significant impact on the design of new treatment regimens with fluoropyrimidines and platinum. Tumors with high TS, TP and DPD expression levels should be treated with non-TS directed anticancer drugs such as CPT-11 or oxaliplatin, or in combination with 5-FU. Patients with high expression of ERCC1 should be treated with non-platinum based regimens whereas patients with low levels would be good candidates for cisplatin or oxaliplatin. With the development of new effective anticancer drugs such as CPT-11 and oxaliplatin, it is of clinical significance to better understand the metabolism and the mechanism of resistance of these new active agents. It is essential to understand why some patients develop life-threatening toxicity and why some tumors are resistant to CPT-11 or oxaliplatin.

213

Pharmacogenetic determinants of toxicity and response in acute lymphoblastic leukemia

M.V. Relling[1,2]. [1]*St Jude Children's Research Hospital, Department of Pharmaceutical Science, Memphis, USA;* [2]*University of Tennessee, Memphis, USA*

Childhood acute lymphoblastic leukemia (ALL) has long served as a model for a drug-responsive cancer. Using chemotherapy alone, event-free survival rates of 80% have been achieved. However, there remain subtypes of ALL with more refractory disease, and these high cure rates are not without serious long-term risks. Our work has focussed on trying to improve cure rates for ALL while minimizing serious adverse effects of therapy, which include second cancers, avascular necrosis of the hip, neurotoxicity, cardiotoxicity, sterility, severe obesity, and hepatotoxicity. Interindividual differences in susceptibility to these adverse effects are due to an interplay between the manner in which treatment is delivered and the underlying susceptibility of the host. Germline polymorphisms in genes responsible for the disposition, metabolism, and responsiveness of the host and of the leukemic blasts are likely to account for some of host variabil-

ity. Our group has generally taken a target-gene approach, genotyping children with ALL whose therapy has been well-documented for polymorphisms of known functional consequence and exploring whether genotypes predict drug-induced phenotypes of interest. For example, we have shown that polymorphisms in thiopurine methyltransferase (TPMT) are associated with an increased frequency of thiopurine-induced acute myelosuppression among ALL patients with at least one mutant allele for TPMT, and this has translated into reduced dosage requirements for such patients. In addition, such patients also appear to be at a higher risk of therapy-induced cancers, such as topoisomerase II-inhibitor-associated secondary AML and irradiation-induced brain tumors. A polymorphic repeat in the thymidylate synthase (TS) gene has been linked to overall event-free survival in childhood ALL. Polymorphisms in glutathione transferase have been associated with ALL outcome in some studies but not others. Although some prior studies linked polymorphisms in CYP3A4 and NQO1 with the risk of secondary myeloid leukemia, among children with ALL, we found no associations between CYP3A4*1B, CYP3A5*3, or NQO1 genotypes and the risk of secondary myeloid malignancies. Relatively modest differences in the delivery of therapy and in study design may account for some of the conflicting conclusions in the literature. Well-controlled clinical trials are required to evaluate the importance of pharmacogenetic variability to to ALL treatment outcomes.

Thursday 21 November

PLENARY SESSION 6

Proffered Papers 2

214

The role of poly(ADP-ribose) polymerase-1 (PARP-1) in the cellular response to topoisomerase I poisons

L. Smith[1], E. Willmore[2], Z. Hostomsky[3], S.E. Webber[3], C.A. Austin[2], N.J. Curtin[1]. *[1]University of Newcastle upon Tyne, Cancer Research Unit, Newcastle upon Tyne, United Kingdom; [2]University of Newcastle upon Tyne, Biochemistry & Genetics, Newcastle upon Tyne, United Kingdom; [3]AgouronPfizer GRD, La Jolla, USA*

DNA Topoisomerase I (Topo I) catalyzes the breakage, unwinding and religation of DNA, forming a transient Topo I-associated DNA strand break (cleavable complex). Topo I poisons, such as camptothecin (CPT) and topotecan (TP), stabilise the cleavable complex, resulting in persistent DNA breaks. PARP-1 is activated by DNA strand breaks and facilitates their repair. PARP-1 inhibitors enhance Topo I poison-induced cytotoxicity[1] but the underlying mechanism has not been defined. Potential mechanisms are: a) PARP-I modulates Topo I activity and b) PARP-1 participates in the repair of Topo I-induced DNA lesions. To elucidate the role of PARP-1 in Topo I poison cytotoxicity we have investigated the effect of a novel potent PARP-1 inhibitor, TBI-361 (Ki<5 nM), in combination with CPT and TP, in human leukaemia cells (K562) and PARP-1 -/- and +/+ mouse embryonic fibroblasts (MEFs). TBI-361 augmented CPT-induced growth inhibition in K562 cells (16 hour exposure): the GI_{50} (growth inhibitory IC_{50}) of 4 $\pm$ 0.6 nM for CPT alone was reduced to 2.4 $\pm$ 0.1 nM by co-incubation with TBI-361. PARP-1 -/- MEFs were 3-fold more sensitive to TP (5-day exposure) than PARP-1 +/+ MEFs; GI_{50} 21 and 65 nM, respectively. TBI-361 caused a 3-fold sensitisation of PARP +/+ cells compared to only a 1.4-fold sensitisation in PARP-/- cells. These data confirm both a role for PARP-1 in Topo I poison cytotoxicity and that the cellular effects of TBI-361 are due to PARP-1 inhibition. The level of Topo I cleavable complexes[2] formed after 30 min exposure to CPT was not significantly altered by TBI-361, and preliminary data shows that TBI-361 has no significant effect on Topo I activity. However, DNA strand breaks induced by CPT were increased by ~20% by TBI-361 after 20 hour but not 30 mins exposure. These data are more consistent with the hypothesis that PARP-1 enhances Topo I cytotoxicity by inhibiting DNA repair rather than a direct effect on Topo I activity. However, the possibility that prolonged exposure to a PARP-1 inhibitor may be necessary to modulate Topo I activity cannot be excluded and ongoing experiments are designed to address this hypothesis. Definition of the mechanism of PARP-1 Topo I interactions will be crucial to exploit fully the clinical potential of PARP-1 inhibitors in combination with Topo I poisons for cancer therapy.

References

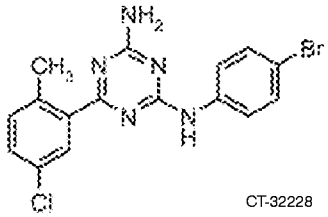
[1] Bowman et al 2001 B J Cancer 84 1 106-112
[2] Padget et al 2000 Biochem Pharm 59 629-638

215

CT-32228, a specific inhibitor of lysophosphatidic acid acyltransferase-beta (LPAAT-b) causes selective tumor cell apoptosis

J.W. Singer, L. Bonham, M. Coon, P. de Vries, D. Hollenback, D. Leung, T. White. *Cell Therapeutics, Inc., Seattle, USA*

Lysophosphatidic acid acyltransferases (LPAATs) are a family of intrinsic membrane enzymes that catalyze the de novo biosynthesis of phosphatidic acid (PA), a co-factor required for raf and mTOR activity. Immunostaining with an isoform-specific monoclonal antibody to LPAAT-b showed strong expression in lung, ovary, prostate, bladder, cervix, and brain tumors while normal tissue expression was primarily limited to endothelial, smooth muscle and inflammatory cells. Ectopic over-expression of LPAAT-b contributed to transformation of NIH/3T3 cells and its removal showed that it was required both for proliferation in low serum and tumor formation in nude mice. Cellular transformation of Rat-1 fibroblasts by Ha-ras led to increased levels of PA and LPAAT activity and increased the amount of 18:1 and 18:2 at the expense of 20:4 fatty acids in cellular lipids, a pattern also seen with LPAAT overexpression. Knockdown of LPAAT-b by RNAi blocked proliferation in DU-145 cells and induced apoptotic cell death in IM9 lymphoblastoid cells. LPAAT-b specific inhibitors were identified following screening of a chemical diversity library. Compounds that inhibited LPAAT-b but not the related housekeeping enzyme, LPAAT-a were selected for optimization. Standard medicinal chemical optimization of hits yielded highly specific structure-activity relationships. Compounds have been identified within 3 classes of related heterocyclics that inhibit LPAAT-b at less than 50 nM in both cell free and intact cell assays. CT-32228, [N-(4-bromophenyl)-6-(5-chloro-2-methylphenyl)-[1,3,5]triazine-2,4-diamine] is representative of one of these classes and is a non-competitive allosteric LPAAT-b inhibitor (Ki 47nM). CT-32228 is anti-proliferative (IC_{50} 50-100nM) and cytotoxic (100-200nM) to a broad variety of tumor cell lines whereas it is not cytotoxic to human hematopoietic progenitors at concentrations up to 2 mM.



CT-32228

In a preliminary study, treating nude mice bearing DU-145 prostate cancer or HT-29 colon cancer with CT-32228 was non-toxic and produced significant tumor growth delay. Similar results were achieved with CT-32548, a follow-on compound with enhanced solubility, in mice bearing NCI-H460 lung cancers. These data suggest that LPAAT-b activity may be critical to oncogenic signaling and potentially represents a novel and selective enzymatic target for cancer therapy that can be inhibited by low molecular weight drug-like compounds.

216

A phase I pharmacokinetic (PK) and serial tumor and skin pharmacodynamic (PD) study of weekly, every 2 weeks or every 3 weeks 1-hour (h) infusion EMD72000, an humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients (p) with advanced tumors known to overexpress the EGFR

J. Tabernero[1], F. Rojo[1], E. Jiménez[2], I. Montaner[2], L. Santomé[1], M. Guix[1], 0. Rosen[3], A. Kovar[3], I. Viaplana[1], J. Baselga[1]. *[1]Vall d'Hebron University Hospital, Medical Oncology Department, Barcelona, Spain; [2]Merck Farma y Química S.A., Barcelona, Spain; [3]Merck KGaA, Darmstadt, Germany*

EMD72000 is an humanized monoclonal antibody directed at the EGFR that has shown potent antitumor activity in preclinical studies. In prior studies EMD72000 has been administered weekly and the MTD has been established at 1,600 mg weekly (Proc. ASCO 2002;38(A378)). In terms of QoL and compatibility with standard chemotherapy schedules a prolongation of the administration interval would be desirable. Insofar, EMD72000 PKs is not linear and the half-life increases with the dose allowing for a more prolonged exposure of the drug. We are therefore conducting a phase I clinical trial of EMD72000 given as a weekly, every 2 or every 3 weeks 1,200 mg 1-hour infusion, with PK and PD assessments to determine the PK profile and