Extended Abstracts

The 16th Meeting of the “Working Group for Pharmacology in Oncology and Hematology (APOH)”

Chairman: PD Dr. K. Mross

Münster, June 21 – 22, 2002

Overview of presentations

G. Hempel, Münster, Germany

The 16th meeting of the CESAR-APOH (Arbeitsgruppe Pharmakologie in der Onkologie und Hämatologie) was held at the Department of Pharmaceutical and Medicinal Chemistry, University of Münster, from June 21 – 22, 2002. The interdisciplinary group APOH consists of clinical oncologists, clinical pharmacologists, clinical pharmacists and chemists who meet once a year to discuss their latest scientific results. The APOH is part of the Central European Society of Anticancer Drug Research (CESAR) and the meeting was a combined workshop and symposium of the APOH together with CESAR which is headed by Prof. Dr. C. Dittrich, Vienna.

The day started with business meetings of different CESAR working groups as were the PHASE I, -II and -III study groups. In the afternoon of the first day the APOH Workshop on “Pharmacogenomics in oncology” started and included 3 lectures given by Prof. Dr. Kroemer, Prof. Dr. Cascorbi and Dr. Zuelsdorf.

Dr. Zuelsdorf gave a general overview showing the expectations regarding pharmacogenomics in oncology and other therapeutic fields. The general hope in the pharmaceutical industry is that geno- and phenotyping patients before therapy can reduce the variability in drug response. This can include polymorphism for drug metabolizing enzymes, differences in receptors or other polymorphisms. Especially in oncology, one hopes to identify responders and patients who will not respond before therapy so that non-responders can receive other therapies and unnecessary side effects be circumvented.

Prof. I. Cascorbi gave a lecture on the genetic factors during carcinogenesis. Several enzyme systems have been identified responsible for the metabolism of xenobiotics showing genetic polymorphisms. These polymorphisms can influence individual susceptibility for cancer either by enhancing the cancer risk or protection against cancer, for example by faster elimination of carcinogens or better DNA repair.

Prof. Kroemer focused on the high-dose chemotherapy with busulfan and showed that there is an upregulation of several genes. They also went a step further and identified proteins which are upregulated during therapy. These findings could help to understand the side-effects of busulfan like liver toxicity.

On the morning of the second day, the business meeting of the APOH took place. The status of CESAR was described by Prof. Dr. Dittrich, president of CESAR, and he discussed the perspectives of this new society within Europe. Furthermore, a new chairman and vice-chairman were elected because PD Dr. K. Mross has held this position now for 2 periods (a total of 5 years; 3 and 2 years). According to the standing orders of the APOH the vice-chairman is the “chairman elect” in case the chairman retires. Prof. Dr. U. Jaehde (Bonn) was confirmed as chairman for the next period 2002 – 2004. Dr. Ralf Hilger (Essen) was nominated and elected for the position of the vice-chairman of the APOH for the same period. Chairman and vice-chairman are ex officio members of the scientific board of CESAR and will have opportunities to discuss issues important for the APOH e.g. co-operations with other groups of CESAR during the regular meetings of this board 3 times a year.

After the business meeting the “Symposium of CESAR-APOH” started and members of the APOH presented new scientific results 13 oral presentations. A broad spectrum of topics was covered by the different speakers. Topics were drug monitoring, new drugs,
surrogate markers and interaction studies. The extended abstracts here summarize the content of 11 (from 13) lectures. After the regular symposium the APOH project “population pharmacokinetics of doxorubicin and epirubicin” was discussed. The recruitment phase (> 500 patients) as well as the analytical phase (> 3200 single analyses) of the PPK project has been terminated and the high amount of data will now be evaluated with the software NONMEM. The specialists busy on this, Dr. Müller (Marburg) and Dr. Port (Heidelberg) had a lively discussion with their colleagues e.g. Dr. Hilger, Prof. Scheulen (Essen) and others.

The APOH is open for all scientists working on pharmacological aspects in oncology and hematology. Further information regarding membership can be obtained from the chairman Prof. Dr. U. Jaehde, Pharmazeutisches Institut der Universität Bonn, An der Immenburg 4, D-53121 Bonn, E-Mail: u.jaehde@uni-bonn.de. Further informations to CESAR can be seen via internet: www.cesar-ewiv.org.

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Molecular-epidemiological aspects of carcinogenesis: the role of xenobiotic metabolizing enzymes

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Introduction

There is increasing evidence that gene-environment interactions play a significant role in an individual’s risk to develop particular smoking related cancers. Despite high penetrance genes, having a low prevalence in the population such as BRCA1 or BRCA2, meta-analysis revealed that common polymorphisms in drug-metabolizing enzymes such as arylamine N-acetyltransferase 2 or glutathione S-transferase M1, and to a less extent cytochrome P450 1A1, may moderately modulate the risk of lung or bladder cancer. Moreover, myeloperoxidase, transforming benzo[a]pyrene and aromatic amines to highly reactive intermediates, ran into focus of molecular-epidemiological studies.

Arylamine N-acetyltransferases

In humans, N/O-acetylation of aromatic and some heterocyclic amines are mediated by 2 arylamine N-acetyltransferases, NAT1 and NAT2, which both exhibit significant genetic polymorphisms. Rapid acetylators turned out to be under-represented in bladder cancer patients with a history of smoking or risk occupation [Brockmöller et al. 1996], but appear to be more frequent among colon cancer patients. Homozygotes have been found to be associated with the risk of lung and laryngeal cancer. It is assumed that NAT1 contributes to the formation of highly reactive acetoxysterases within the bladder epithelium. We could show that the most common allelic variant NAT1*10 obviously does not alter ex vivo p-aminobenzoic acid acetylation, but is significantly less frequent among bladder cancer patients (odds ratio 0.65). Combination with NAT2 revealed that cases carrying NAT1*10 and NAT2*/slow alleles have a 6-fold risk for bladder cancer if they are exposed to hazardous compounds at their place of work [Cascorbi et al. 2001].

Cytochrome P450 1A1 and glutathione S-transferase M1

Polycyclic aromatic hydrocarbons may be bio-activated to mutagenic carcinogens by cytochromes P450 1A1 and 1B1, which are regulated by the Ah receptor. A meta-analysis showed a weak influence of hereditary polymorphism, however we could show in a large study on 324 patients and 298 controls a 3-fold over-representation of carriers of the CYP1A1*2B-allele [Cascorbi et al. 1996]. Other newly detected polymorphisms of CYP1A1 were not associated to lung cancer. Although the I462 amino acid replacement in CYP1A1*2B did not change kinetics towards diol-formation [Schwarz et al. 2001], interestingly, in lung tissue and in lymphocytes, DNA-adducts could be found significantly increased in CYP1A1*2 carriers who had also inactive GSTM1 [Rojas et al. 2000].

Myeloperoxidase

A frequent G/A-polymorphism, located 463 bp upstream of exon 1 in the promoter region, strongly reduces MPO mRNA expression. The highly active variant is significantly overrepresented among lung and laryngeal cancer patients [Cascorbi et al. 2000] and was found to lead to enhanced benzo[a]pyrene diol-epoxide DNA adducts in skin of coal tar treated patients. The molecular-epidemiological results are confirmed by several other studies, suggesting that myeloperoxidase possibly plays a major role in aromatic hydrocarbon metabolism than CYP1A1.
Conclusions

Genetic susceptibility to cancer is based on different levels, like carcinogen activation, detoxification, membrane transport, DNA-repair, tumor suppressor and oncogenes, modulation of cell cycle and apoptosis. The current knowledge on hereditary variances depicts only some small pieces of a mosaic. However, hypothesis driven investigations on genes with a clear role in e.g. xenobiotic metabolism with well-characterized hereditary traits contribute to understand the etiology of cancer and allow risk estimations for a population.

References


Figure 1. Gene-gene environment interaction and risk of bladder cancer: In a case-control study (n = 374/295), cases carrying NAT2/slow and NAT1*10 genotypes are compared to controls with NAT2/rapid and NAT1*4 (Figure according to Cascorbi et al. [2001]).
Aminopterin-human serum albumin conjugate (AP-HSA): uptake and cytotoxic effects in tumor cell lines*

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**Introduction**

The antifolate aminopterin (AP) was synthesized by Seeger et al. [1947] and showed temporary remissions in a clinical trial by Farber et al. [1948] in the treatment of children with acute leukemia.

Its strong toxicity leads to severe side effects, for this reason aminopterin was replaced by methotrexate (MTX). A current study with AP in patients with refractory malignancies by Ratliff et al. [1998] shows encouraging results, but the handling in chemotherapy remains difficult due to its narrow therapeutic window.

Conjugation of AP to human serum albumin (HSA) may lead to greater selectivity for tumor tissue and reduced unintended side effects and may thereby increase its therapeutic potential.

A human serum albumin conjugate with MTX (MTX-HSA) is in successful clinical phase II testing [Hartung et al. 1999, Vis et al. 2002]. In an animal study with Walker-256 carcinoma bearing rats by Kremer et al. [2002], the AP-HSA conjugate showed high antitumor activity in vivo and a favorable toxicity compared to low-molecular-weight AP.

Apart from reducing side effects due to improved tumor accumulation [Wunder et al. 1998], albumin conjugates show a plasma half-life in the range of native albumin which is 19 days [Hartung et al. 1999] and overcome transport resistance as shown by Weigand et al. [2001] in a MTX-resistant clone of CCRF-CEM cells. Because the loading rate determines tumor targeting properties [Stehle et al. 1997] we used AP-HSA covalently linked in a 3.5:1 molar ratio in cytotoxicity testing on rat glial cells (C6) in vitro. We also investigated the uptake of fluorescently labeled albumin into various cell lines by confocal scanning laser microscopy.

**Material and methods**

**Synthesis of HSA conjugates**

The synthesis of MTX-HSA [Stehle et al. 1997], AP-HSA [Kremer et al. 2002] and amino fluorescein (Afl-HSA) [Sinn et al. 1996] were performed as described. AP-HSA and MTX-HSA were purified of low molecular weight contaminants on PD10 columns (Pharmacia) with 0.17 M NaHCO₃ according to the manufacturers specifications. Purity was checked by HPLC on a Zorbax G250 gel-filtration column, solvent 0.02 M sodium citrate pH 7.5, detection 300 nm, flow 1 ml/min. The content of unconjugated MTX in MTX-HSA was determined to be 0.11% and that of AP in AP-HSA to be 0.14%.

**Cell culture model**

C6 (rat glial tumor), A240286S (human lung adenocarcinoma, established by C. Granzow DKFZ) and RPMI 8226 (human multiple myeloma) were grown in RPMI 1640 standard medium supplemented with 10% fetal bovine serum, 4 mM l-glutamine and 25 mM HEPES. C6 cells and A240286S cells grow adherent, and were passaged every 3 – 4 days at a cell density of 1x10⁵ cells/ 75 cm² (A240286S), 1x10⁶ cells/ml (C6) every 2 – 3 days. RPMI 8226 cells grow in suspension and were subcultivated at a cell density of 2x10⁶ cells/ml every 3 – 4 days. Incubation of all 3 cell lines was at 37°C in an atmosphere with 5% CO₂ without addition of antibiotics.

**Cytotoxicity testing**

C6 cells were seeded into 96-well plates at a density of 1x10⁴ cells/100 μl culture me-
After 24 h, AP-HSA or MTX-HSA were added in 100 μl medium at the concentrations indicated in Figure 1. Cell growth was evaluated after further 24 h, 48 h and 72 h by a cell proliferation assay (Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay/ Promega). Absorbance was recorded at 490 nm in an ELISA plate reader.

C6, A240286S or RPMI 8226 cells were seeded into 6-well plates and incubated for 6 hours. Culture medium was drawn off, and replaced by Afl-HSA in fresh medium at a final concentration of 50 μg Afl/ml. After 24 h (C6 and A240286S cells) or 48 h (RPMI 8226 cells) Lyso Tracker Red (Molecular Probes) was added to the medium in a final concentration of 50 nM. After 30 – 45 min cells were washed several times with PBS and inspected by confocal scanning laser microscopy by H. Spring (Biomedical Structure Analysis Group, DKFZ, Heidelberg).

Results and discussion

Cytotoxicity testing

The concentration of AP-HSA resulting in 50% growth inhibition (IC_{50}) was calculated to be 4.68 μg (after 48 h) and 2.77 μg (after 72 h) of AP in the conjugate/ml in C6 cells (Figure 1). MTX-HSA treated cells show comparable effects at more than double the concentration of MTX (8.68 μg of MTX in the conjugate/ml) after 72 h (data not shown). At the IC_{50} concentration, the AP-HSA is contaminated with 3.88 ng AP/ml. As seen in Figure 1, 10 ng AP/ml was only mildly cytotoxic. Therefore the toxicity elicited is solely due to the AP-HSA conjugate. The same is true for MTX-HSA (data not shown), where the unconjugated MTX was even less toxic.

To show that indeed the albumin conjugates are taken up into the cell by endocytosis and are localized in the lysosomes, all 3 cell lines, the murine C6 cells and the human A240286S and RPMI 8226 cells, were incubated with Afl-HSA and Lyso Tracker Red. Colocalization was clearly visible in C6 cells and was more than 80% in the 2 human cell lines as shown for A240286S in Figure 2.

Both A240286S and RPMI 8226 cells were extremely sensitive to AP and therefore testing of AP-HSA toxicity is difficult in these cells. Human melanoma cell lines have, however, been shown to be sensitive to AP-HSA with IC_{50} values in the range of the murine cells C6.

References

ERK1/2 phosphorylation: a biomarker analysis within a phase I study with the new Raf kinase inhibitor BAY43-9006


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Introduction

Ras is a small GTPase that is activated by a variety of cell-surface receptors, such as the Receptor Tyrosine Kinases (RTKs). Activated Ras (GTP-bound) initiates several signaling cascades that are involved in cell cycle regulation. The best characterized is the Raf/MEK/MAPK cascade. Ras initiates this pathway by recruiting Raf kinase to the plasma membrane where it is further modified for full activation. Mutations of the Ras oncogen has been linked to tumor progress and correlated to increased risk of recurrence and death. The incidence of Ras mutations in solid human tumors are given in Table 1. Antisense and dominant negative experiments have demonstrated that eliminating Raf kinase function reverses the Ras transformed phenotype. Here we present a brief report of a method for the determination of phosphorylated (activated) ERK1/2 as a possible biomarker in a phase I study for a small molecule inhibitor of Raf kinase, the Raf-1 inhibitor BAY 43-9006.

Patients

BAY 43-9006 dose regimens were investigated based on 2 strategies:

Strategy 1: increasing the daily BAY 43-9006 dose of a 1-day per week regimen.
Strategy 2: increasing the number of days treated.
BAY 43-9006 was administered orally for at least 4 weeks, but treatment was continued as long as it was beneficial for the patients. Safety evaluations were performed prior to the next dose step.

Patients received 100 mg, 200 mg, or 400 mg on Day 1 of a once-weekly cycle. To test the effect of multiple dosing, 100 mg BAY 43-9006 was also administered as follows:
- b.i.d. at 0 and 6 hours on Day 1 of a once-weekly cycle
- t.i.d. at 0, 6, and 12 hours on Day 1 of a once-weekly cycle
- b.i.d. on Days 1 – 2 of a once-weekly cycle
- b.i.d. on Days 1 – 4 of a once-weekly cycle
- b.i.d. on Days 1 – 7 of a once-weekly cycle
(continuous dosing cohort).

Pharmacodynamics

Blood was withdrawn into EDTA-container, lymphocytes were isolated using a density gradient centrifugation method (d = 1.077). Aliquots of either the whole blood or isolated lymphocytes were stimu-
lated in the presence (under treatment) or absence (prior treatment) of the Raf-1 inhibitor. For validation experiments, the tosylated form of the inhibitor was used, BAY 54-9085. For stimulation of the cells, phorbol myristate acetate (PMA) was used. The stimulated cells were divided for FACS and Western blot analyses, respectively. FACS was performed using dual color expression of CD7 (PE) and goat anti-rabbit IgG (FITC) against phospho-p44/42 MAP Kinase (Erk1/2).

### Results

Up to now, an overall of 66 patients were treated within this study (Table 2). At a dose level of 200 mg b.i.d. of BAY 43-9006, the stimulated response to PMA was inhibited after 3 weeks of treatment. The effect of the drug was more significant at the dose level of 400 mg b.i.d. and the inhibition was highly significant after 11 days of treatment at the 600 mg b.i.d. dose level.

### Conclusion

A biomarker assay for the detection of the activated ERK1/2 protein in peripheral blood lymphocytes (CD7 positive) was established using FACS methodology. There is evidence for an inhibition of the Raf kinase pathway in patients during treatment with the Raf-1 inhibitor BAY43-9006. The obtained effect seems to be more pronounced with higher dosages. The correlation of these results with the PK parameters and patients outcome (toxicity and response) is under investigation.
Interindividual differences in oxaliplatin pharmacokinetics

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Introduction

Oxaliplatin is a platinum complex which has substantial antitumor activity in patients with advanced colorectal cancer when combined with fluorouracil and calcium folinate [De Gramont et al. 2000, Schmoll and Cassidy 2001]. It is known to induce peripheral neuropathy which is characterized by a transient acute dysesthesia and a cumulative distal neurotoxicity [Gent and Massey 2001, Quasthoff and Hartung 2002]. The aim of this study was to investigate the interindividual variability of oxaliplatin pharmacokinetics and to detect patient-specific factors affecting pharmacokinetic parameters as a basis for dosage individualization.

Methods

We analyzed platinum concentrations in total plasma, ultrafiltered plasma and erythrocytes in patients with advanced colorectal cancer treated with 50 mg/m² oxaliplatin given as i.v. short infusion over 2 hours in combination with 2000 mg/m² fluorouracil (over 24 hours) and 500 mg/m² calcium folinate (over 2 hours). Each cycle consisted of 4 drug administrations at Day 1, 8, 15, and 22. Each patient was examined once in the first and once in the second cycle.

Up to now, 6 patients were studied. Table 1 shows the individual pharmacokinetic parameters of oxaliplatin determined in ultrafiltered plasma (UF) in the first cycle of 2 patients receiving first-line and 4 receiving second-line therapy. Platinum clearance was found to be highly correlated to creatinine clearance calculated using the Cockcroft and Gault formula (Figure 1).

Platinum concentration-time profiles differed considerably between plasma and erythrocytes.
In all patients, the AUC in erythrocytes was considerably higher in the second treatment cycle (55.4 ± 15.8 μg x h/ml) compared to the first one (24.9 ± 13.5 μg x h/ml) while AUC in ultrafiltered plasma was only slightly increased (2.91 ± 2.03 μg x h/ml in the second cycle, 2.36 ± 0.77 μg x h/ml in the first cycle). This result indicates that oxaliplatin accumulates in erythrocytes during chemotherapy.

Conclusions

The pharmacokinetic parameters of oxaliplatin exhibit large variability. Our preliminary evaluation suggests no difference between first-line and second-line therapy. Total clearance is significantly related to renal function. This correlation may be used for dosage individualization. The extent of accumulation in erythrocytes differs among individual patients. Future studies will reveal whether erythrocytes may serve as surrogate cells in order to predict cumulative toxicity of oxaliplatin.
Penetration of capecitabine and its metabolites into malignant and healthy tissue of patients with advanced breast cancer*

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Aim of the study

Capecitabine is an oral prodrug of 5-fluorouracil (FU), which is activated mainly in liver and tumor via a cascade of 3 enzymes. Since FU concentrations achieved in malignant lesions are an important determinant of efficacy, we investigated the intratumoral transcapillary transfer of capecitabine and its metabolites in vivo in patients with breast cancer. Using the microdialysis technique, the pharmacokinetics of capecitabine and its metabolites were assessed in the extracellular extravascular space.

Patients and methods

Ten patients with skin metastases from breast cancer received a daily dose of 2500 mg/m² capecitabine administered orally in 2 divided doses for 2 weeks followed by a 1-week rest period. To evaluate the transcapillary transfer of capecitabine, microdialysis probes were inserted into a cutaneous metastasis and subcutaneous connective tissue of capecitabine naive patients. Capecitabine and its metabolites 5'-deoxy-5-fluorocytidine (DFCR), 5'-deoxy-5-fluorouridine (DFUR), and FU were analyzed in plasma and interstitial tissue fluid by capillary electrophoresis.

Results

After peroral administration of capecitabine, high concentrations of the metabolites DFCR and DFUR (mean Cmax: 5.9 μg/ml and 3.8 μg/ml, respectively) were observed in plasma, whereas FU rarely exceeded 0.5 μg/ml plasma (Table 1). Capecitabine and its metabolites easily penetrated malignant and healthy tissue and equilibrated within 30 minutes between plasma and tissue interstitium.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Parameter</th>
<th>DFCR</th>
<th>DFUR</th>
<th>FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Cmax (μg x ml⁻¹)</td>
<td>5.9 ± 0.7</td>
<td>3.8 ± 0.5</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>tmax (h)</td>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>AUC₀-₅h (μg x ml⁻¹ x h)</td>
<td>10.4 ± 1.2</td>
<td>6.6 ± 0.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Tumor</td>
<td>Cmax (μg x ml⁻¹)</td>
<td>4.7 ± 0.7</td>
<td>2.1 ± 0.4</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>tmax (h)</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>AUC₀-₅h (μg x ml⁻¹ x h)</td>
<td>9.7 ± 2.1</td>
<td>4.3 ± 1.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>s.c. Tissue</td>
<td>Cmax (μg x ml⁻¹)</td>
<td>4.6 ± 0.9</td>
<td>2.0 ± 0.5</td>
<td>0.47 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>tmax (h)</td>
<td>2.5 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>AUC₀-₅h (μg x ml⁻¹ x h)</td>
<td>9.4 ± 2.0</td>
<td>3.7 ± 1.0</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>
In the malignant lesion, the exposure to capecitabine was significantly higher when compared with plasma (AUC in tumor vs. plasma: \( p = 0.01 \)), whereas the AUC of all other metabolites did not differ significantly between malignoma and plasma. Comparing the interstitium of subcutaneous connective tissue with plasma, only the AUC of DFUR was significantly lower in healthy tissue (\( p = 0.014 \)). There was, however, a trend for higher exposure to capecitabine in subcutaneous tissue in a subgroup of 4 patients (ratio \( \frac{AUC_{\text{connective tissue}}}{AUC_{\text{plasma}}} > 2 \)). In 3 patients, drug monitoring was repeated at the end of the first therapeutic cycle. Distribution into tissue and metabolism were not subject to clinically relevant changes under daily exposure to capecitabine.

**Conclusions**

Capecitabine and its metabolites DFCR and DFUR equilibrated within 30 minutes between plasma and tissue interstitium. As intended with the prodrug design of capecitabine, FU was present in low concentrations in plasma and tissue interstitium. Under daily therapy, distribution and metabolism of capecitabine were subject to moderate variations after repeated administration. Thus, there was no evidence of drug tolerance to capecitabine, which may be attributed to pharmacokinetic mechanisms.
Serial measurements of pharmacokinetics, DCE-MRI, blood flow, PET and biomarkers in serum/plasma – what is a useful tool in clinical studies of anti-angiogenic drugs?*

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Introduction

Angiogenesis research has been an important topic since Folkman [1971] has pioneered the importance of new vessel formation necessary for the tumor growth. The vascular system is regulated by several pro-angiogenic and anti-angiogenic factors. In case of a tumor, pro-angiogenic factors are necessary in a very complicated concert of signals involving different receptors and their specific ligands including the intracellular signal pathway cascade to induce new vessels which are necessary for the nutrition of the malignant cell clone.

In case angiogenesis is absolutely necessary for tumor growth there will be a good chance to develop new drugs which interfere with the numerous pro-angiogenic factors, their receptors and the downstream signaling pathways [Mross 2000]. Such drug development has happened during the past 5 years, although no drug has been reached the goal to become a recognized licensed drug. Instead a lot of frustration has been observed due to cancellations of very big clinical trials in phase III (e.g. VEGFR Tyrosine Kinase Inhibitors from SUGEN and Matrix Metalloproteinase (MMP) from BAYER) as well as the disappointing results even in early clinical phase I and II trials.

Methods to get insight into the regulation of vessel formation, the visualization of vessel formation, the function of new vessels, the distribution of anti-angiogenic drugs in different compartments and their implementation into clinical trials

We have to remember that a phase I study is a first study in man, the goal is to describe the side effects as complete as possible and to come up with a recommendation for a safe dose in phase II and III studies. The study should be finished with the lowest possible number of patients, within the shortest time possible. The problem is that the safe dose should also be a dose where anti-angiogenic activity can be expected but how can this dose be determined? From preclinical studies it is known, at which drug concentration a 50% inhibition for e.g. the VEGF-R is observed (Receptor IC50). The design of such study should therefore use a rapid dose escalation scheme including a minimum of patients especially at the lowest dose levels to come into that IC50 range. The complete pharmacokinetics should be measured at least 2 times within a patient to describe the pharmacokinetic parameters Cmax/min, AUC and Clp precisely. The classical PK analysis allows to get an impression if one can reach the IC50 known from preclinical studies.

To get some insight into the behavior of serum biomarkers like VEGF, FGF which are pro-angiogenic markers, measurements of such markers should be implemented [Drevs et al. 2001].

To get some information about the leaky tumor vessels it is possible to use the fact that gadolinium, a contrast enhancing drug for MRI measurements, is penetrating the extracellular space via the leaky tumor vessels very fast. Using a dynamic contrast enhanced MRI measurement technique (DCE-MRI) it seems to be possible to get some insight into the permeability of the tumor vessels [Morgan et al. 2001]. The distribution of Gd from the intravascular space to extracellular space and the redistribution back into the vessels lumen can be described by a pharmacokinetic model using the data from a dynamic picturing of the tumor region of interest (ROI). The Gd PK calculations can be performed by a pixel by pixel evaluation of the ROI. Precise blood flow measurements within the tumor vessels are clinically not yet possible because there is no contrast enhancing drug licensed which stays in the vessel (such drug have a large molecular weight like e.g. albumin or iron which cannot penetrate the extracellular space).

Precise blood flow measurements in man are in principle possible with Doppler sonographic examinations of tumor vessels [Drevs et al. 2001].
et al. 2002a]. The problem with this technology is still its dependence from experienced user. The interindividual variability of different investigators is high so longitudinal measurements are severely hampered by this fact. Furthermore only tumors in the abdomen, at best in the liver can be carefully watched in case a vessel is detectable in the tumor metastasis. For very special circumstances e.g. repetitive measurements in hepatocellular cancers this technique can be helpful to get insight to changes in the tumor blood flow during anti-angiogenic therapy.

Tumor-neoangiogenesis and enhanced glucose metabolism are possibly activated by the same stimulus: hypoxia. In a recent published study no correlation between FDG-activity and microvesSEL density (MCV) in the tumor was observed [Veronesi et al. 2002]. Glucose uptake and tumor-neoangiogenesis appear to be independent biological features. Serial FDG-PET scans are possibly not useful for the follow-up of patients treated with anti-angiogenesis drugs.

Results from anti-angiogenic phase I drug trials

Classical PK and the evaluation of the toxicity are the backbone of each phase I trial even in anti-angiogenic research. Biomarkers like serial VEGF measurements are only helpful if the signal is changing in a dose-dependent way in those patients where the tumor remains stable or shrinks. The most attractive technique for valuable signals is the DCE-MRI for drugs, which have influence on the new developing tumor vessels. In case of changing the permeability of the new tumor vessels either by reducing/destroying them by toxic agents or by influencing the VEGF-VEGFR system by specific drugs, signals measured by DCE-MRI should be altered. This was actually observed in case of drugs interfering with the VEGFR [Dreves et al. 2002b, Morgan et al. 2001]. Serial color Doppler sonographic examinations are restricted to very special circumstances which are in general not present in phase I trials due to the very heterogeneous patient population.

Conclusions

In conclusion, the evaluation of anti-angiogenic drugs within clinical phase I trials should use the classical instruments: (a rapid) dose escalation scheme, evaluation of the toxicity profile, evaluation of the pharmacokinetics and metabolism of the study drug, a limited number of biomarkers like VEGF and FGF and as a new tool the dynamic contrast enhanced MRI technique which gives signals that can be related to tumor vessel permeability by pharmacokinetic evaluation of the gadolinium distribution. Besides this, classical size measurements of the tumor can be done very precisely by the MRI technique too. Thus, classical evaluation of the tumor response using the RECIST and WHO criteria is possible. This can be refined by the evaluation of the apparent diffusion coefficient (ADC) of a tumor’s water content which is a measure of necrosis [Dzik-Jurasz et al. 2002]. All these instruments are available. Up to now, there are no results which support the hypothesis, that the dose – anti-angiogenic activity (dose-effect) curve is bell-shaped. In other words, there is no need to change the principal concept of phase I studies to escalate the dose up to the dose-limiting toxicities. The most important additional tool which allows meaningful pharmacodynamic evaluations (dose-effect; PK parameters-effect) is the MRI technique.

References


The influence of elevated liver function parameters on the pharmacokinetics of doxorubicin and epirubicin – a population based pharmacokinetic study of CESAR-APOH*

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Introduction

The liver is the most important organ for metabolism of anticancer agents, irrespective whether administration of the drug is by oral or intravenous route. Therefore, consideration of liver function is very important if drugs with mainly hepatic inactivation and elimination are used [Pratt and Kaplan 2000]. Damage of liver cells is represented by elevated concentrations of aspartate-ammonotransferase (AST) and alanine-aminotransferase (ALT), whereas an increase in bilirubin can be seen with intrahepatic cholestase. Serum concentrations of these parameters are used for estimation of hepatic function. Because increased toxicity of some drugs can be seen with loss of liver function capacity, dose reduction of the anthracyclines doxorubicin and epirubicin is recommended in dependence on the serum concentrations of AST and bilirubin. There are no reliable data for calculation of the amount of dose reduction, however, but only recommendations based on clinical experiences [Mross 1993].

Therefore, a prospective multicenter clinical trial on patients with different cancers with and without hepatic metastasis was initiated. The patients were treated with doxorubicin (DOX) or epirubicin (EPI) as indicated. This was a project of the APOH, a working group of the “Central European Society for Anticancer Drug Research (CESAR)”. Because only a limited number of samples could be taken from each cancer patient, and for identification of special subgroups with delayed elimination, a population pharmacokinetic examination was intended. Special interest focused on the influence of liver metastasis on the metabolism of the anthracyclines. Doxorubicinol and epirubicinol are metabolites with anticancer activity, thus the examination of the concentrations of these metabolites in addition to the parent drugs was included, together with the parameters sex, body weight, and serum concentrations of AST, ALT and bilirubin.

Recruitment was finished after inclusion of 524 patients, with 203 patients on treatment with doxorubicin and another 321 patients treated with epirubicin. A first preliminary population based pharmacokinetic examination was performed on the patients treated with doxorubicin, and the results of this examination are presented here.

Patients and methods

This subexamination was performed on 203 patients (76 male, 123 female, 4 unknown sex) treated with doxorubicin (DOX). The age range was 16 – 87 years (mean 52, median 53). The absolute dose of doxorubicin was 18 – 150 mg (mean 73.6 mg, median 72.5 mg) with an infusion time ranging from 1 – 2697 min (mean 54.2 min, median 12.5 min). Plasma concentrations of the parent drug and of doxorubicinol were available from 3 different time points from each patient. The samples were taken during the time intervals from 0 – 30 min, 30 – 120 min, and 120 min to 48 h after administration of the drug, for the first, second and third sample, respectively.

Determination of plasma concentrations was performed by HPLC with solid phase ex-
traction and fluorescence detection [Mross et al. 1988].

For population based examination of the pharmacokinetic data, the Nonlinear Mixed Effects Model Program (NONMEM), version V, level 1.1 developed by Stuart Beal and Lewis Sheiner (University of California, San Francisco) was used.

**Results**

The data set showed a considerable heterogeneity, especially in regard to the great range in infusion times. In addition, during the examination it was obvious, that by transmission of the data from the clinics, a relevant number of errors were included into the data set, which have to be eliminated. Therefore, before realization of these limitations, attempts to perform a common examination on DOX together with the metabolite DOXol was not successful. Hence, examinations limited on the data of the parent drug (DOX) were performed, using 1-, 2- and 3-compartment models. A 2-compartment model including the influence of female sex and of the bilirubin plasma concentrations on the doxorubicin clearance obtained the best fit. A correlation between DOX concentrations measured and the estimations of the computer program is shown in Figure 1.

A comparison of the estimated DOX clearances with the bilirubin serum concentrations and the presence of liver metastasis showed a correlation between these parame-

![Correlation between measured doxorubicin plasma concentrations and estimations on the basis of a 2-compartment model.](image)

**Figure 1.** Correlation between measured doxorubicin plasma concentrations and estimations on the basis of a 2-compartment model.

![Correlation between clearance (CL), bilirubin (BILI) plasma concentrations and filiae (FIL).](image)

**Figure 2.** Correlation between clearance (CL), bilirubin (BILI) plasma concentrations and filiae (FIL). For data estimation the presence of liver metastasis was encoded by the number "1", whereas patients without hepatic filiae were encoded by the "0".
ters as depicted in Figure 2. In the group of patients with the lowest DOX clearances the highest bilirubin concentrations were measured, and the greatest elimination rate of doxorubicin was observed in patients without liver metastasis.

Conclusion

Drugs metabolized and eliminated mainly by the liver are difficult to administer in patients with reduced liver function because of possibly enhanced toxicity. Thus, a dose reduction is recommended. There are, however, no clear algorithms for dose adjustment in cancer patients with hepatic filiae treated with anthracyclines. Recent results suggested that current dose reductions based solely on the extent to which bilirubin is elevated may not be optimal [Twelves et al. 1998]. To get more insight, a clinical study with DOX and EPI including more than 500 patients with different malignant diseases was performed to develop a pharmacokinetic-based dose reduction schedule. Also at present only preliminary results can be presented, a correlation between the reduction in anthracycline clearance, elevated bilirubin serum concentrations and hepatic filiae can be demonstrated. The evaluation of all data is still ongoing and will include gender, Bili, AST, ALT, AP, Alb and presence of liver metastasis. This population based pharmacokinetic examinations including these covariables will be performed on the entire data set for a definition of a dose recommendations in patients with impaired liver function, and altered liver biochemistry respectively treated with the 2 anthracyclines DOX and EPI.

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Population pharmacokinetics of etoposide

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Introduction

Etoposide, a semisynthetic podophyllotoxin derivative, plays an important role in the treatment of several malignancies [Joel 1996]. Numerous studies have demonstrated a large interindividual pharmacokinetic variability of etoposide probably contributing to the variation in tumor response and toxicity. A population pharmacokinetic analysis can be performed to identify patient-specific measurable factors that influence the pharmacokinetic disposition of etoposide. Integrating these factors in a population model may help predict individual pharmacokinetic parameters and hence facilitate dosage individualization.

The aim of this study was to investigate the pharmacokinetics of etoposide in a heterogeneous patient population using a population pharmacokinetic approach and to quantify the effect of dose and various patient-specific factors on etoposide pharmacokinetics.

Patients and methods

A total of 60 patients (17 females, 43 males) suffering from different types of tumors were studied. They were treated with etoposide as part of their chemotherapy regimen with doses ranging from 50 – 1450 mg. Etoposide was administered as short intravenous infusion (duration ranging from 30 min to 1 hour). In 12 patients, concurrent treatment with ifosfamide was part of the chemotherapy regimen. Serial blood samples were drawn (2 – 6 per patient) up to 24 h after the end of infusion and plasma samples were analyzed for etoposide by a reversed-phase HPLC method [Farina et al. 1981, Stremetzne et al. 1997].

A 2-compartment model was assumed for pharmacokinetic parameter estimation. Pharmacokinetic data analysis was performed using the software P-Pharm (Simed S.A., Créteil, Cedex, France). Maximum likelihood estimates were computed by using a 2-step iterative algorithm (EM-like algorithm) [Gomeni et al. 1994]. The influence of potential co-variates including etoposide dose and concurrent treatment with ifosfamide was assessed by using multiple linear regression models. Initial population parameter estimates for clearance (CL), volume of distribution in the central compartment (Vc), and the transfer rate constants k12 and k21 were obtained from those patients with an adequate number of concentration time-points (n = 27).

Results

A total of 244 plasma samples was included in the population pharmacokinetic analysis. The parameter estimates without and with integration of co-variates into the population model are given in Table 1. CL increased significantly with increasing creatinine clearance. In addition, concurrent treatment with ifosfamide was found to increase etoposide CL. Vc was found to be significantly correlated with body surface area and etoposide dose, and inversely correlated with serum albumin concentration. The coefficients of variation of CL and Vc characteriz-
ing the residual unexplained variability could be decreased from 25 to 14% and from 20 to 13%, respectively, by including these co-variates in the population pharmacokinetic model.

Conclusions

In conclusion, the variability of the pharmacokinetic parameters $CL$ and $V_c$ could be related to certain co-variates. By including these co-variates in the population pharmacokinetic model, the residual unexplained variability of the pharmacokinetic parameters was considerably reduced. The predictive power of the population pharmacokinetic model will have to be evaluated by either cross validation or a prospective application before it can be used for dosage individualization.

References


Results of phase I pharmacokinetic and pharmacodynamic studies of the Raf kinase inhibitor BAY 43-9006 in patients with solid tumors*

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Introduction

Ras is a small GTPase that is activated by a variety of cell-surface receptors. Activated Ras initiates several signaling cascades that are involved in cell cycle regulation. The best characterized is the Raf/MEK/ERK cascade. Ras initiates this pathway by recruiting Raf kinase to the plasma membrane where it is further modified for full activation. Mutations in the Ras gene have been documented for several tumor types. Specifically, 50% of colorectal carcinomas, 90% of pancreatic carcinomas, and 20% of hematopoietic malignancies contain Ras mutations that activate the Raf/MEK/ERK kinase signaling pathway. In addition, breast, ovarian, and prostate carcinomas have growth factor receptor mutations that lead to Ras signaling. Antisense and dominant negative experiments have demonstrated that eliminating Raf kinase function reverses the Ras transformed phenotype. BAY 43-9006 is a novel potent, orally active inhibitor of Raf-1 and the first compound in this class to enter clinical trials. The primary objectives of the presented studies are to: define dose limiting toxicities (DLTs) and maximum tolerated dose (MTD), determine the pharmacokinetic (PK) profile, and describe evidence of anti-tumor activity and inhibition of ERK1/2 phosphorylation in treated patients (pts).

Patients and methods

To date, 163 patients in the phase I clinical program have been treated with BAY 43-9006 according to the 4 different dosing schedules.

- 1. Continuous dosing schedule: Study 100283 (Essen, Germany).

Doses included: 50 mg, 100 mg, 200 mg, 400 mg, 600 mg, 800 mg.

Schedules included: 1 dose on Day 1 of a weekly cycle; 2 doses on Day 1 of a weekly cycle; 3 doses on Day 1 of a weekly cycle; b.i.d. on Days 1 – 2 of a once-weekly cycle; b.i.d. on Days 1 – 4 of a once-weekly cycle; b.i.d. on Days 1 – 7 of a once-weekly cycle (continuous dosing cohort).

- 2. Schedule 28 days on/7 days off dosing schedule: Study 100277 (Ontario, Canada).

- 3. Schedule 21 days on/7 days off dosing schedule: Study 10164 (Brussels, Belgium).

- 4. Schedule 7 days on/7 days off dosing schedule: Study 100342 (Boston, MA, USA).

PK parameters were calculated with KinCalc(c) using a compartment-free approach.

Results of pharmacodynamic studies are presented separately (R.A. Hilger et al.).

Results

Two confirmed partial responses have been observed at dose level 400 mg b.i.d. continuous treatment (HCC) and 600 mg b.i.d. 21 d on/7 d off (RCC). Five additional patients achieved tumor shrinkage ≥ 20%; 10 additional patients have SD > 6 months. Toxicities were generally mild to moderate. Dose-limiting-toxicity (DLT) was diarrhea (grade III CTC) at dose level 800 mg b.i.d. DLT at dose level 600 mg b.i.d. continuous and 600 mg b.i.d. 21 d on/7 d off was reversible skin toxicity ( rash, palmar-plantar erythema). BAY 43-9006 plasma Cmax/AUC val-
ues increased upon multiple dosing indicative of accumulation. These increases were generally consistent with steady state $C_{\text{max}}/\text{AUC}$ values based on linear pharmacokinetics. Dose-proportionality of BAY 43-9006 pharmacokinetics is likely up to 400 mg b.i.d. PK profiles (0 – 12 h), obtained at start of treatment and steady state (after day 7), were $\text{AUC}_{0\rightarrow12\text{h(ss)}} = 73 \text{ mg} \times \text{ h/l}$, $C_{\text{max}} = 9.9 \text{ mg/l}$, and $t_{\text{max}} = 1.75 \text{ h}$ at 400 mg b.i.d. Given the high interpatient PK variability, it was not possible to discern increases in $C_{\text{max}}$ and $\text{AUC}_{(0-\tau)}$ past the dose of 400 mg b.i.d.

### Conclusions

BAY 43-9006 is a new Raf kinase inhibitor that is generally well-tolerated using continuous oral dosing. There is preliminary evidence for an inhibition of the Raf kinase pathway in patients treated with BAY 43-9006. This effect appears to be more pronounced with higher dosages. Clinical studies with BAY 43-9006 in combination with other chemotherapy agents are in progress. Phase II studies are planned.

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Monitoring of methotrexate and reduced folates in the cerebrospinal fluid of cancer patients

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**Introduction**

Methotrexate (MTX) inhibits the enzyme dihydrofolate reductase leading to a decrease in levels of reduced folates such as 5-methyltetrahydrofolate and 5,10-methylenetetrahydrofolate. After high-dose infusions of MTX calcium folinate is given as rescue medication. One of the proposed mechanisms of rescue is the increase of reduced folates pool by the conversion of calcium folinate into 5-methyltetrahydrofolate [Borsi et al. 1990]. The aim of this study was to develop a method for the simultaneous determination of MTX, 5,10-methylenetetrahydrofolate, 5-methyltetrahydrofolate, and calcium folinate in the cerebrospinal fluid (CSF) in order to monitor the effects of MTX and calcium folinate on the concentrations of reduced folates.

**Patients and methods**

A HPLC method for the determination of the analytes of interest in the CSF was developed by adapting a published method [Belz et al. 1994]. The analytes were eluted by using a gradient consisting of a 0.01 M phosphate buffer (pH 2.1) and methanol. Fluorescence detection (294/356 nm) was used for analyzing 5,10-methylenetetrahydrofolate and 5-methyltetrahydrofolate and UV detection (310 nm) for calcium folinate and MTX. CSF samples were collected in vials containing ascorbic acid and centrifuged prior to injection.

To determine the applicability of the described method for monitoring MTX and reduced folates, CSF samples from 2 patients suffering from primary CNS lymphoma were analyzed.

Patient 1 received an infusion of MTX (5 g/m\(^2\)/24 h) and calcium folinate rescue (60 mg every 6 h) which was started 1 hour after the end of MTX infusion. Samples were taken from the Ommaya reservoir at the end of the MTX infusion, and 1, 3.5, 7 and 24 h after the beginning of calcium folinate rescue.

Patient 2 received chemotherapy according to a protocol for primary CNS lymphoma consisting of 2 cycles (1 and 2) with 3 blocks (A, B, and C) each. The duration of one block

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**Table 1. Validation parameters of 5,10-methylenetetrahydrofolate (5,10-methylene THF), 5-methyltetrahydrofolate (5-methylTHF), calcium folinate and methotrexate in the CSF.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linear conc. range (ng/ml)</th>
<th>Coefficient of correlation</th>
<th>Precision (CV, %)</th>
<th>Accuracy (%)</th>
<th>Limit of detection (ng/ml)</th>
<th>Limit of quantification (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,10-methyleneTHF</td>
<td>10 – 150</td>
<td>0.99953</td>
<td>6.9 – 14.7</td>
<td>88 – 112</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>5-methylTHF</td>
<td>10 – 150</td>
<td>0.99998</td>
<td>3.9 – 10.5</td>
<td>91 – 112</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Calcium folinate</td>
<td>50 – 2500</td>
<td>0.99999</td>
<td>1.3 – 6.7</td>
<td>78 – 103</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>10 – 5000</td>
<td>0.99997</td>
<td>1.3 – 8.8</td>
<td>78 – 103</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

CV = coefficient of variation, THF = tetrahydrofolate
was 5 – 7 days. In block A and B, the patient received an infusion of MTX (5 g/m2/24 h) at Day 1 and intrathecal MTX (3 mg) at Days 2, 3 and 4. Calcium folinate rescue was started between Days 2 and 3. In block C, the patient did not receive systemic MTX and calcium folinate. Intrathecal MTX (3 mg) was given at Days 3, 4, 5 and 6. CSF samples were obtained from the Ommaya reservoir every day before the administration of intrathecal MTX.

Results

In order to validate the HPLC method linearity, precision, accuracy and the limits of detection and quantification were assessed. The results were in accordance with international criteria for validation of bioanalytical methods (Table 1).

In the samples from Patient 1, 5-methyltetrahydrofolate and 5,10-methylenetetrahydrofolate were below limit of quantification at the end of the MTX infusion. 7.5 h after the start of calcium folinate rescue, the levels of 5,10-methylenetetrahydrofolate and 5-methyltetrahydrofolate were 14 ng/ml and 11 ng/ml and rose to 22 ng/ml and 14 ng/ml, respectively, 24 h after the start of rescue while the concentration of calcium folinate was 59 ng/ml. MTX levels decreased from 1306 ng/ml (start of rescue) to 99 ng/ml (24 h later).

In the samples from Patient 2 in blocks A1, B1 and B2, the levels of 5-methyltetrahydrofolate could be quantified from 24 hours after the end of the MTX infusion onwards. At Day 5, 5-methyltetrahydrofolate levels were 12.4 ng/ml, 30 ng/ml and 37 ng/ml in blocks A1, B1 and B2, respectively. In blocks C1 and C2, 5-methyltetrahydrofolate levels decreased during intrathecal MTX therapy from 29 ng/ml at Day 3 to 14 ng/ml at Day 6 (C1) and 34 ng/ml at Day 3 to 22 ng/ml at Day 6 (C2). During the same time period, MTX levels increased. 5,10-methylenetetrahydrofolate could not be quantified in this patient.

Conclusions

The results suggest that our method is suitable for monitoring methotrexate and reduced folates in the CSF of cancer patients. Monitoring of these analytes could help optimize the calcium folinate rescue in patients receiving high-dose methotrexate and give some insight into the effects of intrathecal methotrexate when no calcium folinate rescue is concomitantly given.

References

Serum HER-2/neu: a new predictive marker*

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The HER-2/neu molecule

Activation and overexpression of cellular oncogenes are considered to play an important role in the development of human cancer. One important oncogene is the human epidermal growth factor receptor 2, also known as HER-2/neu and commonly referred to as c-erbB-2.

The HER-2/neu oncogene encodes a transmembrane tyrosine kinase growth factor receptor that is expressed on cells of epithelial origin. The HER-2/neu molecule consists of 3 domains: an internal tyrosine kinase, a short transmembrane section, and an extracellular ligand binding domain that is referred to as the extracellular domain (ECD).

The ECD is a glycoprotein with a molecular weight between 97 and 110 kD that can be released from the cell surface by proteolytic cleavage and become accessible for measurement in serum.

Methods for analysis

Currently the most widely used methods to assess the HER-2/neu-status of a tumor are immunohistochemistry (IHC) to detect the HER-2 protein and fluorescent in situ hybridization (FISH) to detect gene amplification. We introduced a double sandwich ELISA using 2 monoclonal antibodies that is available in the microtiter plate format and on the Immuno-1 automatic analyzer. This assay can be used to measure the full-length HER-2/neu molecule in tumor homogenates and the circulating ECD in serum. The assay has been approved by the FDA to monitor serum ECD in IHC-positive patients under therapy.

Whereas FISH and IHC are routinely used to determine the status of a tumor at the time of primary surgery, the ELISA offers an opportunity to continuously monitor ECD in patients serum.

Clinical value of HER-2/neu in breast cancer

Numerous studies using either IHC or ELISA have shown that the HER-2/neu oncoprotein is overexpressed in approximately 20 – 40% of women with breast cancer. Therapy with Herceptin (Trastuzumab, Genentech Inc., South San Francisco, CA, USA) is directed against the extracellular domain of HER-2/neu and approved for administration alone or in combination with chemotherapies to women with metastatic breast cancer that overexpresses the HER-2/neu protein.

Currently the standard test to determine if a breast tumor displays elevated HER-2/neu is IHC, in case of equivocal results a FISH test is performed.

The tissue blocks used to determine whether a woman is eligible for Herceptin treatment usually had been taken at the time of primary surgery. Amounting evidence is now published that a major proportion of tumors that initially had been HER-2/neu-negative can convert and form HER-2/neu-positive metastases. Clinical efficacy of Herceptin therapy has been demonstrated in this patient population. Therefore the initial IHC and FISH analyses do not always accurately reflect the HER-2/neu status of metastases. The single most important limitation to tissue testing, the general unavailability of repeat assessments, may therefore result in suboptimal treatment of patients that had HER-2/neu-negative primary tumors but whose metastases are HER-2/neu-positive.

In contrast, blood samples to measure the levels of circulating HER-2/neu-ECD can be obtained repeatedly and the molecule is easily detectable in the serum of breast cancer patients.

Studies have shown that breast cancer patients with elevated serum levels of HER-2/neu have a poor prognosis and shorter survival. It has been shown that the tumors of these patients grow more aggressively than tumors in breast cancer patients who do not have elevated serum levels of the HER-2/neu ECD.
About 50% of women with breast cancer develop distant metastases within 5 years of primary detection. Early detection of metastatic disease is an essential prerequisite for successful therapy. Therefore, tools that can be used to detect recurrence before the appearance of clinical signs can become crucial to the long-term survival of these patients.

Many reports have now established that HER-2/neu serum levels increase with tumor burden and that monitoring the HER-2/neu serum levels post-surgery provides an important tool to detect recurrence early. Several studies have suggested that if women have elevated HER-2/neu tissue expression or elevated serum levels of the ECD at the time of initial diagnosis, they should be monitored post surgery for increasing levels of ECD. Some reports indicate that elevated HER-2/neu levels post-surgery may be an indicator for micrometastases.

It is also common that women who did not overexpress HER-2/neu at the time of primary diagnosis will develop elevated serum HER-2/neu levels during the course of disease. An incremental rise of serum ECD usually precedes clinical manifestation of metastases.

**Conclusion**

While many factors, e.g. hormone receptor status, ploidy and growth fraction, as well as the expression of various oncogenes and protooncogenes by the tumor cells have added value to therapeutic decision-making, there is still a need for improvement of the management of breast cancer patients.

Measuring the HER-2/neu oncogene by IHC, FISH and ELISA allows selection of breast cancer patients for specific treatment with Herceptin.

Monitoring the serum levels of HER-2/neu allows to follow the clinical course and response of patients under therapy.

Cumulating evidence suggests that serum HER-2/neu levels can independently predict response to chemo- and hormonal therapies in breast cancer patients.
Population pharmacokinetics of cyclophosphamide, doxorubicin and etoposide in 30 patients with BEACOPP chemotherapy*


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Introduction

The BEACOPP chemotherapy regimen consists of the substances bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone. It was developed by the German Hodgkin’s Lymphoma Study Group (GHSG) in 1991 and is used in the treatment of intermediate and advanced stage Hodgkin’s disease in clinical trials [Engel et al. 2000]. Therapeutic outcome is superior to the COPP/ABVD regimen but toxicity, mainly hematotoxicity, is considerable. Blood cell nadirs shows great inter- and intraindividual variability. In a study of the GHSG, hematotoxicity has shown to be dose-dependent, but no further factors except gender were found to describe the individual risk for toxicity. Individual pharmacokinetics may be the missing link between dose and toxicity.

Study objectives

– Examination of the pharmacokinetics of drugs given on the first day of BEACOPP therapy (cyclophosphamide, doxorubicin, etoposide).
– Correlation of individual pharmacokinetics to hematotoxicity.
– Determination of possible drug-drug-interactions of the drugs used in the BEACOPP regimen.

Patients and methods

Thirty patients treated with one of the BEACOPP chemotherapy regimens (Table 1) were included in the study. The first day of the first 3 cycles, respectively, was examined. In each cycle, 3 plasma samples were drawn (immediately, approx. 1 hour and 21 hours after the end of all infusions). Blood cell counts were recorded as part of the routine therapy monitoring to determine toxicity. Plasma drug concentrations of cyclophosphamide (HPLC), doxorubicin and doxorubicinol (capillary electrophoresis) and etoposide (HPLC) were determined using validated methods.

Drug concentrations were analyzed using population pharmacokinetic methods (NONMEM V). One- and multi-compartmental models were used. For doxorubicin and its metabolite, a combined model evaluating plasma concentrations of doxorubicin and doxorubicinol simultaneously was developed. Cofactors to be included in the model refinement were therapy cycle, age, gender, body height, body weight, body surface area and body mass index. The discrimination of the best fit was performed using the log-likelihood ratio test (95% significance level).

First, a fixed effect model and an error model were selected. Then, cofactor analysis was performed by multiple repetitive testing and inclusion of the most significant cofactor(s) in the model until a full model was obtained. With this model, exclusion of cofactor(s) was tested, and the error model was re-evaluated.

Total body clearance and volumes of distribution were determined, and the individual AUC was calculated as AUC = dose/ clearance. Data are presented as geometric means and geometric coefficients of variation.

Results

Twenty-one men and 9 women completed 3 cycles and were hence included in the final
analysis. Seven patients received standard doses of BEACOPP (3 of them in a scheme omitting procarbazine), 16 patients received a dose-escalated regimen (with respect to cyclophosphamide, etoposide and doxorubicin) and 7 a time-escalated regimen (recycle at Day 14). Demographic data of the patients are given in Table 1. Doses, infusion times and blood sampling times are summarized in Table 2.

For cyclophosphamide, a 1-compartment model with the cofactors "height" and "cycle" both on clearance and volume of distribution best described the data. Clearance was modelled as $CL = (0.276 \times \text{cycle}1 + 0.285 \times \text{cycle}2 + 0.276 \times \text{cycle}3 - 0.00119 \times \text{body height}) \times \exp(\xi1)$, the volume of distribution as $Vd = (−86 \times \text{cycle}1 − 86 \times \text{cycle}2 − 90.6 \times \text{cycle}3 + 0.75 \times \text{body height}) \times \exp(\xi2)$, where $\xi1$ and $\xi2$ are normally distributed with a mean of 0 and a standard deviation of 0.315 and 0.227 and account for the interindividual variability of CL and Vd, respectively. The geometric mean of the modelled clearance was 72.2 ml/min (CV 26.8%), the Vd averaged 43.8 l (CV 24.7%). CL was estimated to be 10 ml/min higher in cycles 2 than in cycles 1 and 3, Vd was estimated to be 5 l lower in cycle 3. AUC was 0.39 mg/h/ml (CV 34.1%).

A 3-compartment model best described the doxorubicin data (Figure 1). The central volume of distribution showed a positive correlation to body mass index, the volume of distribution of doxorubicinol significantly decreased with age. The metabolic clearance of doxorubicin to doxorubicinol was lower in females, and the residual body clearance of doxorubicin (excluding the biotransformation of doxorubicin to doxorubicinol) was positively correlated to body surface area. AUC was 2.88 mg/h/ml (CV 17.4%).

A 2-compartment model was most appropriate for the description of etoposide plasma concentrations. Central Vd averaged 12.7 l (CV 20.5%), peripheral Vd was 146.7 l (CV 13.7%) with an intercompartment clearance of 15.3 ml/min (CV 44.2%). Total body clearance was 25.7 ml/min (CV 7.4%). Central Vd was positively correlated to age as well as peripheral Vd to body weight. The total body clearance showed an inter occasion variability (with variability decreasing in cycles 2 and 3). AUC was 617 mg/h/ml (CV 35.8%).

Conclusions

Infusion times and consecutively peak plasma concentrations varied considerably between patients. This may influence toxicity, especially regarding doxorubicin [Horthobagyi et al. 1988]. AUC values of cyclophosphamide and etoposide showed considerable inter- and intraindividual variability, while AUC values of doxorubicin varied with doses.

Table 1. Demographic data of 30 patients treated with various BEACOPP protocols.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.6</td>
<td>17 – 59</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>176.7</td>
<td>156 – 196</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74.0</td>
<td>53.5 – 99</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.91</td>
<td>1.58 – 2.26</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7</td>
<td>18.3 – 33.3</td>
</tr>
</tbody>
</table>

Table 2. Doses, infusion times and blood sampling times (mean, range) of cyclophosphamide, doxorubicin and etoposide given on the first day of the first 3 cycles of BEACOPP chemotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Cyclophosphamide</th>
<th>Doxorubicin</th>
<th>Etoposide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg)</td>
<td>1770 (1040 – 2700)</td>
<td>57 (40 – 75)</td>
<td>279 (160 – 434)</td>
</tr>
<tr>
<td>Duration of infusion (min)</td>
<td>65 (11 – 178)</td>
<td>35 (2 – 95)</td>
<td>68 (5 – 213)</td>
</tr>
<tr>
<td>1st blood sampling (min)</td>
<td>139 (27 – 403)</td>
<td>129 (5 – 485)</td>
<td>140 (5 – 405)</td>
</tr>
<tr>
<td>2nd blood sampling (min)</td>
<td>221 (94 – 470)</td>
<td>208 (60 – 579)</td>
<td>226 (100 – 950)</td>
</tr>
<tr>
<td>3rd blood sampling (min)</td>
<td>1325 (960 – 1530)</td>
<td>1275 (1018 – 1482)</td>
<td>1330 (888 – 1572)</td>
</tr>
</tbody>
</table>
Pharmacokinetic parameters of cyclophosphamide were comparable to those seen in previous pharmacokinetic studies [Busse and Kroemer 1997].

The parameters for doxorubicin may not be directly compared to data from the literature, because to date no combined models of doxorubicin and doxorubicinol are published. Despite these limitations, doxorubicin clearance was similar to previous studies [Piscitelli et al. 1993]. The central volume of distribution observed in the present study was slightly lower, but taken into account the great interindividual variability, this may be of minor importance. The peripheral Vd was lower as well, but for this value data in literature differ considerably. Because of the sparse data situation, individual variability could not be examined for all parameters of the model; the inclusion of variability for peripheral Vd and doxorubicinol clearance showed no improvement of model fit.

Etoposide pharmacokinetics were comparable to literature [Nguyen et al. 1998], with the exception of peripheral Vd for which much lower volumes of distribution were described.

We were initially able to fit another 2-compartment model with a lower peripheral Vd to the data, but in the model refinement process, the model shown above proved to be more robust.

Thus, the drugs given as part of the BEACOPP regimen seem to have no relevant influence on the pharmacokinetics of the 3 drugs examined.

Several influence factors for the pharmacokinetic parameters could be identified during the model building process. Because the study was not primarily designed for the examination of these influence factors, further studies should be carried out to examine whether these influence factors are suitable for therapy individualization.

Further analyses of the data will be done: The models will be refined including parameters of hepatic and renal function as cofactors. Pharmacokinetics of procarbazine and prednisone (which are also part of the BEACOPP regimen) will be examined. A population-based model of thrombocyte kinetics following the administration of chemotherapy will be applied to the data.

With these models, the examination of the second study aim, the link between individual pharmacokinetics and toxicity, will be possible.

Acknowledgments

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