NCy 03**DETECTION, SEPARATION AND EXTRACTION OF DOXORUBICIN, 4'-EPI-DOXORUBICIN AND SEVEN METABOLITES FROM PLASMA SAMPLES**

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Epirubicin, the 4'-epimer of doxorubicin, is now introduced into the clinic and phase II and phase III studies are in progress. This doxorubicin analog seems to have a similar cytostatic potency as doxorubicin but a reduced pattern of side-effects. Especially cardiotoxicity is reduced. It is known that the pharmacokinetics and metabolism of epi-DOX differs from that of DOX. For sophisticated pharmacokinetic studies (e.g. DOX versus epi-DOX) it is essential to use a reliable extraction, separation and detection technique to quantify the parent drugs (epi-DOX and DOX) and their metabolites in biological fluids.

We developed an extraction method with high recoveries for all metabolites using a solid - liquid extraction procedure. For the most polar metabolites, the glucuronides, a recovery of 90 - 100 % was found. For all other metabolites (doxorubicin aglycone (Aone), 7-deoxy Aone (7d-Aone), epi-doxorubicinol (Eol), doxorubicinol aglycone (Aolone) and 7-deoxy Aolone (7d-Aolone)) and the parent drugs (epi-DOX and DOX) recoveries from 60 - 90 % were calculated.

It was possible to separate all metabolites and the parent drugs using a reversed phase HPLC system and a sensitive fluorescence detector. The two glucuronides (epi-DOX glucuronide (E-glu) and epi-doxorubicinol glucuronide (Eol-glu)) could be detected down to 0.2 ng/ml plasma whereas the 7d-compounds can be monitored down to 1 ng/ml plasma. All other metabolites and the parent drugs could be detected within this range. Isocratic elution resulted in complete separation of all metabolites within nine minutes. This procedure provides a reliable tool for comparative pharmacokinetic studies on different anthracyclines.

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NCy 04**IN VITRO AND PHASE I CLINICAL STUDIES WITH 7-CON-O-METHYLNAGARIL (MENOGARIL).**

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Menogaril is a semisynthetic anthracycline prepared from *Streptomyces nogater* which has significant antitumor activity in a variety of murine tumor model systems. We have conducted *in vitro* and *in vivo* studies with Menogaril in order to determine potentially sensitive tumor types as well as toxicity in humans. Menogaril was tested in a human tumor cloning system against 288 tumor specimens from 28 different tumor types. At 0.02 µg/ml the drug was active in 20 of 70 evaluable specimens (29%). Breast, colorectal, and non-small cell lung cancer specimens were most sensitive. Subsequently 24 patients in a phase I clinical trial received 54 courses of Menogaril with doses ranging from 16 to 256 mg/m². The drug was administered as an i.v. infusion over 60 min every 21 days. Granulocytopenia was dose-limiting and severe at high doses. Other side effects included hair loss and phlebitis at injection sites. Transient atrial fibrillation was noted in one patient. The maximally tolerated dose was 256 mg/m². The following pharmacokinetic data were derived from 14 patients: t 1/2 alpha: 0.05 h, t 1/2 beta: 1.76 h, t 1/2 gamma: 25.9 h, Vd: 816.1 ± 299.6 l/m², mean total body plasma clearance: 22.7 ± 3.55 l/h/m². Average peak plasma concentration at the maximum tolerated dose was 877 µg/ml (n = 2). The observed data were best described by a three compartment model with zero order infusion input and first order elimination. Renal excretion did not appear to be important for the elimination of the drug. Phase II studies are recommended in patients with breast, non-small cell lung, and colorectal cancer, with starting doses of 160 mg/m² for patients with prior antitumor treatment and 200 mg/m² for patients without pretreatment.

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NCy 05**CHEMISTRY OF NEW DERIVATIVES OF 4-HYDROXYCYCLOPHOSPHAMIDE**
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Cyclophosphamide (CP) is metabolized in the liver to 4-hydroxy-CP which is a highly unstable compound. Our chemical approaches for stabilisation involved the substitution of the C-4-position by a sulfoalkylthio-moiety or by a hydroxyurea-group. Within these new classes of compounds mafosfamide (cis-4-(2-sulfoethylthio)-CP) was evaluated in clinical studies. The stereochemistry of mafosfamide and other oxazaphosphorines in solid form and in solution was confirmed by X-ray diffraction and by application of phosphorus decoupling in H,H-COSY-NMR-spectra, a novel tool for stereoanalysis of oxazaphosphorines as well as of other phosphorus compounds which are important biomolecules.

Under physiological conditions mafosfamide isomerizes to its trans-epimer, equilibrating at a 59:41-ratio within less than 5 minutes. Simultaneously a rapid initial hydrolysis occurs to 4-hydroxy-CP and 2-mercapto-ethane-sulfonate. The lowest hydrolytic speed of mafosfamide was observed about pH 4.

Analytical approaches for the determination of mafosfamide and its metabolites are TLC, HPLC and photometry. Above all FAB-MS-spectra analysis is useful for the identification and quantification of mafosfamide or of the conjugate of activated ifosfamide with mesna in urine.

Preliminary results with 4-hydroxyurea-CP derivatives have shown that these compounds are biological highly active and have a significantly reduced toxicity in comparison to mafosfamide. Dissolution in water leads to a slow hydrolysis to 4-hydroxy-CP and hydroxyurea with a half-life-time of about 20 hours at pH 7, 37°C.

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NCy 06**PHASE-I-TRIAL OF TUMOR-NECROSIS-FACTOR IN PATIENTS WITH MALIGNANT DISEASE.**

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Tumor necrosis factor (TNF) is a cytokine produced by the monocyte-macrophage-system in mice treated with BCG and challenged with endotoxin. TNF causes hemorrhagic necrosis in certain experimental tumors and seems to be without effect on non-malignant cells. Recently the complementary DNA of human TNF could be successfully cloned. The recombinant human TNF (rHuTNF) was tested in preclinical investigations. After the completion of the preclinical tests we started a phase-I-trial for the evaluation of toxicity of rHuTNF (Asahi Chemicals) in patients with malignant disease whose tumors were resistant to conventional chemotherapy or radiotherapy. TNF in the plasma of the patients was assayed immunologically and functionally by ELISA and the L-cell assay. The initial dose was 1 x 10⁵ U/m². If no severe toxicity was observed in three consecutive patients at a certain dose level, the dose was gradually escalated. To date 4 patients received intratumoral injection of rHuTNF. Up to a dose of 2 x 10⁵ U/m² the only side effects were chills and fever up to 40°C. So far, no measurable levels of rHuTNF could be detected in these patients after intratumoral injection by ELISA nor by the L-cell assay.

The recruitment for the phase-I-study of rHuTNF continues and additional information will be provided on intratumoral and intravenous application in patients with different types of malignant disease.

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