44

Cytochrome P450 3A4 mediates transformation of methoxymorpholinyl doxorubicin (Nemorubicin; MMDX) to its highly potent metabolite PNU-159682 in human liver microsomes

L. Quintieri[1], M. Fantin[1], R. Battaglia[2], A. Rosato[3], M. Caruso[2], C. Geroni[2], P. Zanovello[3], M. Floreani[1]. [1]*Pharmacology Section, Dept. of Pharmacology and Anesthesiology, University of Padua;* [2]*Oncology, Pharmacia Corporation, Nerviano (MI), Italy;* [3]*Oncology Section, Dept. of Oncology and Surgical Sciences, University of Padua, Padua, Italy*

MMDX is a promising doxorubicin (DX) derivative currently undergoing clinical investigation for treatment of hepatocellular carcinoma. Despite an *in vitro* cytotoxicity comparable or slightly (2-10-fold) higher than that of DX, MMDX is 50-100-fold more potent than DX *in vivo* both in experimental animals and in humans. Furthermore, our previous *in vivo* studies carried out in the mouse model suggest that an MMDX metabolite(s) possessing a higher potency than the parent compound and synthesized via cytochrome P450 3A (CYP3A) mediates MMDX activity *in vivo* (Quintieri et al., Cancer Res.; 60: 3232, 2000). Among the known metabolites of MMDX, only PNU-159682, a compound we previously isolated from an incubation mixture of the drug with NADPH-fortified rat liver microsomes, shows significantly higher *in vitro* cytotoxicity compared with MMDX; its biosynthesis may therefore explain the high potency of MMDX *in vivo*. The aims of this study were to evaluate the ability of human liver microsomes (HLM) to catalyze conversion of MMDX to PNU-159682 and to identify the enzyme(s) involved. Preliminary studies using 14C-MMDX and radio-HPLC analysis indicated that biotransformation of MMDX by HLM is an NADPH-dependent process and that PNU-159682 represents the major liver microsomal metabolite of the drug. Further experiments were conducted using HPLC with fluorescence detection for quantitative evaluation of metabolite formation. Kinetic analyses indicated that a single enzyme supports conversion of MMDX to PNU-159682. CYP3A4 appears to be responsible for PNU-159682 synthesis based on the following key results: 1) among the CYP inhibitors tested, only TAO (100 μM) and ketoconazole (1 μM), both of which are selective inhibitors of CYP3A enzymes, strongly inhibit PNU-159682 formation (85 and 95% inhibition, respectively); 2) metabolite formation is dose-dependently inhibited by a monoclonal anti-CYP3A4/5 antibody; 3) using 10 individual preparations of human liver microsomes, we observed a highly significant correlation (r2 = 0.97) between the rate of PNU-159682 formation and testosterone 6 β-hydroxylase activity, a marker of CYP3A; 4) only microsomes from CYP3A4 cDNA-transfected lymphoblastoid cells but not those from cells engineered to express other human liver CYP proteins are able to catalyze the formation of detectable amounts of the metabolite. In conclusion, PNU-159682 represents a CYP3A4-generated hepatic metabolite of MMDX in humans.

45

Preclinical activity against liver metastases of Nemorubicin, a DNA-intercalating cytotoxic agent for the treatment of hepatocellular carcinoma

C. Geroni[1], L. Quintieri[2], O. Valota[1], A. Rosato[3], P. Zanovello[3], M. Floreani[2], F. Sola[1], M.A. Pacciarini[1]. [1]*Pharmacia Corporation, Oncology, Nerviano (MI), Italy;* [2]*Pharmacology Section, Dept. of Pharmacology and Anesthesiology,* [3]*Oncology Section, Dept. of Oncology and Surgical Sciences, University of Padua, Padua, Italy*

Nemorubicin (3'desamino-3'[2(S)methoxy-4-morpholinyl]doxorubicin-hydrochloride, PNU-152243) is a highly lipophilic anthracycline currently under clinical development for the intra-hepatic artery treatment of hepatocellular carcinoma. This anticancer drug is biotransformed in the liver into a more cytotoxic metabolite(s) by means of a cytocrome P4503A (CYP3A)-dependent process, as shown *in vitro* by incubating the drug with NADPH-fortified liver microsomes (Quintieri et al, 2000). The metabolism of Nemorubicin is not fully elucidated; at present one metabolite exhibiting higher potency compared to the parent compound has been identified (Geroni et al, 1997). The metabolic potentiation of Nemorubicin by hepatic microsomal enzyme(s) may contribute to rendering this drug highly active against primary liver tumors or liver metastases. A phase I study reported regressions in patients with liver metastases from colorectal cancer (Vasey et al, 1995). We studied the activity of Nemorubicin in comparison to doxorubicin (DX) in C57BL/6 mice bearing tumors derived from the cell line M5076, which preferentially metastasize to the liver. Unlike DX, Nemorubicin is more effective against liver metastases than against the primary solid tumor following i.v. administration. Results showed an increase in lifespan (ILS) of 59% and 67% for Nemorubicin and 63% and 39% for DX on primary tumor and metastases, respectively. Moreover, Nemorubicin shows higher antimetastatic activity following oral administration (ILS, 107%); this finding supports a role of an active metabolite(s) synthesized via intestinal and/or hepatic enzyme(s) in its activity toward tumor cells growing in the liver. The role of CYP3A-mediated Nemorubicin metabolism on its *in vivo* antitumor activity and toxicity has been tested. Pretreatment of mice with the prototypical inducers of CYP3A, pregnenolone-16β-carbonitrile (PCN) and dexamethasone (DEX), increases liver microsomal potentiation of the *in vitro* cytotoxicity of Nemorubicin. Furthermore, pretreatment of animals with DEX reduces Nemorubicin toxicity, while not interfering with its antitumor activity. Conversely, administration of troleandomycin, a selective inhibitor of CYP3A activity, markedly inhibits the antitumor activity of Nemorubicin and increases drug tolerability. Collectively, these findings suggest that a Nemorubicin active metabolite(s) synthesized by CYP3A contributes significantly to its antitumor activity, mainly at the hepatic level.

46

Cellular pharmacology of PNU-159682, a liver microsomal metabolite of methoxymorpholinyl doxorubicin (Nemorubicin; MMDX)

L. Quintieri[1], E. Rossi[2], D. Saggioro[2], G. Pennella[3], C. Geroni[3], J. Lobo Antunes[4], B. Gatto[4], M. Floreani[1], P. Zanovello[2]. [1]*Pharmacology Section, Dept. of Pharmacology and Anesthesiology, University of Padua;* [2]*Oncology Section, Dept. of Oncology and Surgical Sciences, University of Padua;* [3]*Pharmacia Corporation, Oncology, Nerviano (MI), Italy;* [4]*Dept. of Pharmaceutical Sciences, University of Padua, Padua, Italy*

PNU-159682 is a major liver microsomal metabolite of MMDX, a doxorubicin (DX) derivative currently undergoing a phase II/III clinical trial. The purposes of this work were to obtain preliminary information about the cytotoxicity, effects on cell cycle progression, and DNA-binding properties of PNU-159682. In a first set of experiments we compared the *in vitro* cytotoxicity of PNU-159682 with that of MMDX and DX toward a panel of eight tumor cell lines including three human leukemias (EM-2, Jurkat and CEM), a human colon adenocarcinoma (HT-29), a human ovarian carcinoma (A2780), a human prostatic carcinoma (DU145), a murine melanoma (B16F10) and a murine leukemia (L1210). Each tumor cell line was exposed to the drugs for 1 h and then cultured in drug-free medium for 72 h. Drug concentrations that decreased cell growth by 70% (IC70) were calculated from dose-response curves by linear interpolation. The IC70 values of PNU-159682 ranged between 0.07 and 0.58 nM; these values were considerably lower than that recorded for both MMDX (67.6-577.9 nM) and DX (181.0-1,717.3 nM). Further experiments analyzed the cell cycle perturbations induced in LoVo colon adenocarcinoma cells by exposure to equitoxic concentrations of each drug. Cells were continuously exposed to the IC70 value of each compound recorded after 72 h of drug treatment, and the cell cycle distribution profiles analyzed using flow cytometry. Exposure to PNU-159682 determined an accumulation of the cells in the S or G2/M phase at 24 h; in contrast, MMDX and DX induced a G1 and a G2/M arrest, respectively. Between 24 and 48 h of treatment a small number of cells started to undergo apoptosis as revealed by the presence of a sub-G1 peak at 48 h. This phenomenon became more evident at 72 h of drug exposure. Preliminary DNA unwinding studies showed that PNU-159682 is able to intercalate into DNA with a higher affinity than MMDX and DX. In conclusion, the results described indicate that PNU-159682 is a compound endowed with a much higher cytotoxicity than MMDX and DX. Furthermore, the different effect of PNU-159682 on cell cycle progression and its higher DNA binding affinity, compared with that of MMDX and DX, suggest that it might possess a different molecular mechanism of action.

47

Properties of the new anthracycline derivative containing modified daunosamine moiety

A. Opolski[1], M. Wasowska[2], J. Wietrzyk[1], A. Nasulewicz[1], I. Oszczapowicz[2], J. Oszczapowicz[3]. [1]*Institute of Immunology and Experimental Therapy, Tumor Immunology, Wroclaw, Poland;* [2]*Institute of Biotechnology and Antibiotics, Warsaw, Poland;* [3]*Warsaw University, Chemistry, Warsaw, Poland*

The anthracycline antibiotics such as daunorubicin, doxorubicin, epidoxorubicin are widely used drugs in the treatment of variety of human neoplastic diseases. However, their clinical effectiveness is limited by several factors, including dose-dependent cardiotoxicity. We have found that during synthesis of new derivatives of daunorubicin with amidine group in 3' position, a new compound (AOX) containing oxazoline ring in daunosamine