moiety was formed. The structure of that one was confirmed by 1H and 13C NMR and IR spectra. Advantageous influence of such modification in daunosamine moiety on the biological properties of AOX, as compared to parental daunorubicin, was observed. Lethal toxicity of AOX is considerably lower (about 20 times). The LD_{50} (lethal dose 50%) of AOX in mice approaches 64.0 mg/kg, whereas the LD_{50} of daunorubicin -3,1 mg/kg. Preliminary histological studies on the cardiotoxicity have shown that cardiotoxicity of AOX is significantly lower than that of parental daunorubicin. It should be stressed that AOX, in contrary to referential daunorubicin, is able to overcome the barrier of drug resistance in *in vitro* conditions. The resistance index (RI) for AOX (ID_{50} value against the cells of anthracycline resistant cell subline divided by respective value against the cells of drug sensitive parental line) is about 1. These results show that antiproliferative activity of AOX against drug sensitive and drug resistant cells is very similar. Moreover, compound AOX revealed the antiproliferative activity *in vitro* against the cells of various mouse and human cancer cell lines and antitumor activity *in vivo* in P 388 mouse leukemia model similar or even higher than referential daunorubicin. Modification in daunosamine moiety (AOX) appeared to cause also important changes in the physicochemical properties. We have observed that stability of AOX during storage in different temperatures as well as in organic solutions is higher than those of parental daunorubicin. It has to be pointed out that the observed results evidently show that such significant modification of the structure of daunosamine moiety in daunorubicin molecule (AOX) does not decrease its antiproliferative activity but induces the advantageous changes of its biological and physicochemical properties such as decreased lethal toxicity and cardiotoxicity, increased stability as well as the ability to overcome the barrier of drug resistance.

48

The combination of nemorubicin with cisplatin and mitomycin C is synergistic in experimental tumor models

C. Geroni, M.A. Pacciarini, O. Valota. *Pharmacia Corporation, Oncology, Nerviano, Italy*

Nemorubicin (3'desamino-3' [2(S)methoxy-4-morpholinyl] doxorubicin-hydrochloride, PNU-152243) is a doxorubicin derivative currently undergoing clinical investigation for the treatment of hepatocellular carcinoma. The compound is characterized by high potency and broad spectrum of antitumor activity, including activity on multidrug resistant (MDR) models, with respect to other anthracyclines. Combination studies have been performed considering both the mode of action of nemorubicin and its ability to overcome MDR (both p-glycoprotein and topoisomerase II related MDR mechanisms) and alkylating agent resistance. Moreover, tumor cells selected for resistance to nemorubicin are specifically resistant to morpholinylanthracyclines and sensitive to all other antitumor drugs. Interestingly, nemorubicin-resistant tumors show a remarkable collateral sensitivity to cisplatin and mitomycin C, with no increase in general toxicity. The model chosen for combination studies is the disseminated L1210 murine leukemia. Mice are treated with nemorubicin (i.v. on day 1 and 2 post tumor implant), or cisplatin or mitomycin C (i.v. on day 3), or their combination. Results: At the highest not-toxic dose (HNTD, 0.06 mg/kg/dose, total dose of 0.12 mg/kg), nemorubicin administered as a single agent shows a 67% increase in life span (ILS). The HNTD of cisplatin alone (10 mg/kg) or mitomycin C alone (6 mg/kg) presents 50% or 0% ILS, respectively Clear synergy is obtained at the highest non toxic combination of nemorubicin (0.06 mg/kg/dose) and cisplatin (10 mg/kg) with a 133% ILS. Synergy is retained on 2 additional lower dose levels. Synergistic antitumor effect is also observed when nemorubicin is combined with mitomycin C. The highest non-toxic combination doses of nemorubicin (0.04 mg/kg/dose) and mitomycin C (4 mg/kg) show a 93% ILS. In conclusion, these data provide a rationale for further clinical trial using the administration of nemorubicin in combination with cisplatin and mitomycin C.

Bioreductive agents

49

A role for over-expressed human cytosolic NOS-II in the bioactivation and toxicity of tirapazamine *in vitro*

E. Chinje, J. Feng, N. Wind, R. Cowen, I. Stratford. *University of Manchester, School of Pharmacy & Pharmaceutical Sciences, Manchester, United Kingdom*

Tirapazamine (TPZ) is a lead member of a class of bioreductive drugs currently in Phase II and III clinical trials. Recent studies have suggested that TPZ radicals that are formed outside the nucleus do not contribute to intranuclear DNA damage and that metabolism that occurs in the cytoplasm is probably irrelevant for activity of this drug in producing DNA-damaging radical. In this study, we have investigated the role of over-expressed cytosolic inducible nitric oxide synthase (NOS II) in the bioactivation of this DNA-damaging antitumour agent. NOS is a dimeric enzyme providing two catalytic domains: an oxygenase and a reductase domain. The reductase domain shares a high degree of sequence homology with cytochrome P450 reductase, known to bioactivate TPZ. To achieve this, we have constitutively over-expressed human NOS-II activity in the breast cancer cell line MDA 231 by employing an optimised expression vector in which the strong human polypeptide chain elongation factor 1 alpha promoter drives a bicistronic message containing the genes for human NOS II and the puromycin resistant gene (pac). In transfected cells, subcellular localization following differential centrifugation and nuclei isolation, showed NOS-II catalytic activity was elevated within the cytoplasm and was exclusively soluble, as determined by conventional activity assay. Both confocal microscopy and Western Blotting studies were carried out to confirm our findings. NOS reductase activity as measured by the NADPH-dependent reduction of cytochrome c ranged from 7.5 to 22.0 nmol cyt. c reduced/min/mg. The highest activity represented about 8-fold increase over parental activity. Similarly, the catalytic activity of the oxygenase domain, measured by the conversion of 14C-labelled L-arginine to L-citrulline, ranged from 20 to 66 pmol citrulline / min/mg and the highest activity was about 110-fold over parental cell activity. NADPH-dependent metabolism of TPZ to the stable two-electron metabolite, SR4317, was determined by HPLC analysis. Increase in rate of metabolism mirrored the elevation in NOS II reductase expression. More significantly, sensitivity to TPZ following 3 hr hypoxic exposure also increased with elevated NOS II reductase levels. Taken collectively, our findings are of significant importance since they indicate that cytosolic NOS II-derived tirapazamine radicals may be important in enhancing its cellular toxicity through DNA damage.

50

A phase I study of fenretinide combined with paclitaxel and cisplatin for the treatment of refractory solid tumors

G. Otterson[1], J. Lavelle[1], M. Villalona-Calero[1], M. Shah[1], K. Chan[2], B. Fischer[1], M. Grever[1]. *[1]Ohio State University Comprehensive Cancer Center, Mediccal Oncology, Columbus; [2]Ohio State University Comprehensive Cancer Center, Pharmacy, Columbus, USA*

Fenretinide (F) is a semi-synthetic retinoid with antiproliferative activity in preclinical studies. F is able to induce apoptosis in a variety of cell types, independent of the expression of retinoid receptors, and appears to function in receptor mediated and non-receptor mediated pathways. Cisplatin (C) and paclitaxel (P) have broad activity alone and in combination in a variety of solid tumors. F has a distinct and interesting mechanism of action and demonstrates more than additive activity when combined *in vitro* with a variety of agents including C and P. The objectives of this study were to determine the maximum tolerated doses and clinical toxicity of C/P/F when used in combination in patients with advanced cancer, and to determine a recommended phase II dose of these agents together. In addition, we characterized the pharmacokinetics (PK) of these agents when administered together and documented responses in patients. All patients provided informed consent for this NCI and IRB approved protocol. C/P were given in standard fashion on day 2, and F was given orally in 2 divided daily doses for 8 days, starting 24 hours prior to C/P. Cycles were repeated every 3 weeks for a maximum of 6 cycles. PK was obtained on all 3 drugs on day 2 (following C/P) and day 8 (following the last dose of F) during cycle one. 15 patients (mean age 57.3, range 26-77) were enrolled on the study, 14 were assessable for PK, toxicity and response. One patient was removed prior to completing the 1st cycle of treatment as he developed acute cholecystitis. The remaining 14 patients received between 1 and 6 cycles of treatment (with 2 patients currently receiving treatment). Dose limiting toxicity (Gr 3 diarrhea and Gr 4 neutropenia) was encountered in 2/4 patients at 80/175/1800 mg/m^2 C/P/F. Seven patients received 2-6 cycles at the next (recommended) dose level of 60/135/1800. NCI CTC 2.0 grade 3 and 4 toxicities included fatigue, nausea/vomiting, neuropathy, and dehydration. 2 patients had a major response (NSCLC and squamous esophageal) and 4 patients had stable disease for up to 6 cycles. A major issue of tolerability was the number of 100 mg pills required to achieve the recommended dose of F. Many patients were unable to swallow the prescribed 26+ daily pills due to nausea or other factors. Compliance with F dosing ranged from 53-100%. PK analysis is underway at the time of submission. (Supported by U01-CA76576-02 and P30-CA16058).