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THE IMPACT OF TIMING OF CHEMOTHERAPY RELATIVE TO DARBEPOETIN ALFA (DA) ON DA PHARMACOKINETICS (PK) AND HEMATOLOGIC EFFECTS. A. C. Heatherington, PhD, D. Henry, MD, R. Patel, MD, N. S. Tchekmedyian, MD, R. Berg, BS, M. Austin, MS, G. Rossi, PhD, J. Glaspy, MD, Amgen Inc, Joan Karnell Cancer Center, Comprehensive Blood and Cancer, Pacific Shores Medical Group, UCLA, Thousand Oaks, CA.

DA is usually administered synchronously with chemotherapy (ctx). This study evaluated whether timing of DA dosing, relative to ctx, impacted its PK and efficacy. The study was conducted in anemic cancer patients receiving ctx once every 3 weeks (Q3W), randomized to receive DA 6.75 µg/kg Q3W either 1 week before ctx (asynchronous) or on the same day as ctx (synchronous) for up to 16 weeks. The effects of cyclic ctx on PK of DA and endogenous EPO levels were evaluated in a sub-set of patients after 1st dose. 81 patients were randomized to receive study drug. Synchronous dosing resulted in higher maximal DA concentrations (mean ± SD) (26.5 ± 9.67 ng/mL, n=13) than asynchronous dosing (15.5 ± 5.25 ng/mL, n=12), with a 1.6-fold increase in exposure (AUC) over week 1. However, DA concentrations increased between 7-10 days post-dose due to ctx administration at day 7 for the asynchronous group, resulting in a 1.4-fold greater AUC for wks 2 and 3. DA terminal half-life tended to be longer in the asynchronous group (87.7 ± 26.0 hr vs 60.9 ± 22.3 hr). In both groups, EPO was elevated for 1 week after ctx, with peak concentrations (4-5-fold increase) at 48 hr. Despite the PK differences, mean (95% CI) change in hgb at wk 7 was similar [0.95 (0.56, 1.33) g/dL vs 1.03 (0.58, 1.47) g/dL]. These results show that DA dosed once per cycle is effective in treating chemotherapy-induced anemia. The findings also indicate that the bone marrow may play a role in the clearance of these agents.

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APOPTOSIS IN ANTI-TUMOR EFFECT OF IRINOTECAN IS MEDIATED BY P53 IN HUMAN HEPATOCELLULAR CARCINOMA CELL LINE. K. Kamio, T. Kumai, PhD, N. Mastumoto, MD, PhD, Y. Takeba, PhD, S. Sekine, MD, R. Taniguchi, MD, S. Kobayashi, MD, PhD, St. Marianna University School of Medicine, Kawasaki, Japan.

Purpose: Frequently used anti-cancer drug irinotecan (CPT11), inhibitor of DNA synthesis, is recently known as to have apoptosis effect, though little is known yet. This study investigated this apoptosis mechanism in human hepatocellular carcinoma cell lines (Huh7).

Methods: The cells were cultured with SN38, active metabolite of CPT11, for 24 h. The cells were analyzed to investigate expression of p53 protein and apoptosis related protein by Western Blotting. In addition to this, another group of cells were cultured with SN38 after p53 antisense (AS) pre-treatment for 24 h and were analyzed by immunocytochemistry for apoptosis related protein evaluation.

Results: SN38 decreased cell survival, and increased expression of p53, caspase-3 and CAD (caspase-activated DNase). Expression of Bcl-xL was suppressed by SN38. On the other hand, in p53 AS pre-treated cells, SN38 decreased expression of p53 protein and apoptosis was suppressed. Caspase-3 expression was induced by SN38 while suppressed by p53 AS+SN38 treatment. Anti-apoptotic protein Bcl-xL was suppressed by SN38 while not by p53 AS+SN38.

Conclusion: This study proved active metabolite SN38 increased p53, apoptosis inducing protein caspase-3, and decreased anti-apoptotic protein Bcl-xL, while these changes were reversed by p53 AS pre-treatment. These results suggest that p53-mediated apoptosis is important mechanism for CPT11 anti-tumor effect on human hepatocellular carcinoma.

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A NOVEL CHICKEN EMBRYO MODEL FOR THE INVESTIGATION OF DRUGS WITH ANTIMETASTATIC PROPERTIES. D. M. Gvozdzan, MD, E. T. Bowden, PhD, A. Wellstein, MD, PhD, Georgetown University, Washington, DC.

The chicken embryo is known to be immunotolerant to the introduction of human cancer cells. We present a newly developed chicken embryo model suitable for the investigation of the metastatic process and for testing drugs with antimetastatic potential.

Fertilized 3 day old chicken eggs were transferred into Petri dishes, and incubated at 37°C. 5-10% of embryos remained viable for 7-10 days. 1×10^5 cells from 3 different breast cancer cell lines were injected on day 5 of embryonic development in the allantoic sac. Stereomicroscopy allowed us to monitor cancer cell movement in real time *ex vivo*. After 12 days, embryos were harvested, and livers were taken for further examination. Using histology, metastases were detected and semi quantitatively evaluated.

We conclude that it is possible to use a chicken embryo as a live model for monitoring and observing the metastatic process, thereby highlighting this system as a unique and novel method for investigation of drugs with antimetastatic properties.

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COMPARISON OF GENOTYPIC AND PHENOTYPIC STRATEGIES FOR INDIVIDUALIZED THERAPY WITH THE NARROW THERAPEUTIC DRUG WARFARIN. V. Michaud, MSc, N. Morin, MSc, D. Brouillette, MSc, D. Roy, MD, L. Verret, MSc, N. Noel, MSc, I. Taillon, MSc, G. O'Hara, MD, D. Gossard, MD, M. Champagne, BSc, M. Vanier, MSc, J. Turgeon, PhD, Université de Montréal, Montréal, Canada.

CYP2C9 metabolizes a wide variety of drugs, including losartan and (S)-warfarin. CYP2C9 is polymorphic and correlation between warfarin clearance and CYP2C9 genotypes has been demonstrated in healthy volunteers and selected patients. The overall objective of our study was to determine, in patients with a multiple drug regimen, correlations between required doses of warfarin and 1) CYP2C9 genotypes, or 2) CYP2C9 phenotype (losartan metabolic ratio). Losartan and its main metabolite EXP 3174 were analysed by HPLC in 6-hour urine samples collected from 77 subjects after a single 12.5 mg oral dose of losartan. The three most common CYP2C9 allelic variants were analysed by PCR-RFLP using genomic DNA of 121 patients. Mean urinary metabolic ratios of losartan were 3.9 ± 3.7 for CYP2C9*1/*1 (n=49), 4.0 ± 5.4 for *1/*2 (n=13), 5.4 ± 4.5 for *1/*3 (n=8), 5.2 for *2/*2 (n=2) and 12.8 for *2/*3 (n=1). A multiple linear regression analysis model was developed using phenotype, age, weight, gender and amiodarone treatment as cofactors. This model explains up to 40% of variability in warfarin dose. In contrast, a genotype analysis correlated with phenotype values only in patients carrying two copies of variant alleles. Our results indicate that in more than 90% of patients, a genotypic approach does not predict required doses of warfarin. On the other hand, CYP2C9 phenotype, when feasible, could represent a more favourable approach to explain intersubject variability in warfarin disposition.