activity. Importantly, inhibition of NFkB with Bay 11-7085 (8.5 mM) leads to loss of cell viability and is accompanied by decreased levels of HSP70 and HSP27, as well as their transcription factor, HSF-1, at the RNA and protein levels. Furthermore, overexpression of a phosphorylation-deficient mutant of IKBa in MIAPaCa-2 cells resulted in decreased NFkB activity and the loss of HSP70 expression.
Conclusion: HSP70 is known to be regulated by several mechanisms. Here we show a novel NFkB-mediated mechanism by which HSF-1 and its transcriptional targets, HSP27 and HSP70 are down-regulated in pancreatic cancer cells in response to triptolide.
Disclosure of Interest: U. Matsathit: None declared. O. McGinn: None declared. V. Sangwan: None declared. S. Banerjee: None declared. L. Phivthong-ngam: None declared. A. Saluja: Other: Chief Scientific Officer, Minneamrita Therapeutics.

PP116—METASTATIC BREAST CARCINOMA INDUCES VASCULAR ENDOTHELIAL DYSFUNCTION IN BALB-C MICE: ROLE OF TUMOR NECROSIS FACTOR-ALPHA AND NADPH-OXIDASE

S. Dalaklioglu[1*]; A. Tasatargil[1]; S. Kale[1]; G. Tanriover[2]; S. Dilmac[2]; and N. Erin[1]
[1]Department of Pharmacology; and [2]Department of Histology and Embryology, Akdeniz University Medical Faculty, Antalya, Turkey
Introduction: Although the oxidative stress and inflammation are closely related with breast cancer, there is no study directly examining the possible changes in vascular functions in the presence of breast carcinoma. The goal of the present study was to evaluate changes in vascular reactivity in tumor-bearing mice.
Patients (or Materials) and Methods: In this study, highly metastatic breast carcinoma cells which was derived from liver or brain metastasis of 4T1 murine breast carcinoma (4TLM and 4TBM, respectively), and 67NR cells which is tumorogenic but nonmetastatic cells were used. Female Balb-c mice 8 to 10 weeks old were divided into following groups: (1) control; (2) injected with 67NR; (3) injected with 4TLM; and (4) injected with 4TBM orthotopically. Thoracic aorta was removed 25 days after injection of tumor cells. Isometric tension studies were performed in response to potassium chloride (KCl), phenylephrine (Phe), acetylcholine (ACh, an endothelium-dependent vasodilator), and sodium nitroprusside (SNP, an endothelium-independent vasodilator). Endothelial nitric oxide synthase (eNOS), phosphorylated eNOS (Ser 1177) (p-eNOS), gp91phox, and tumor necrosis factor-α (TNF-α) expressions in aortic tissues were demonstrated by immunohistochemistry. The level of TNF-α in vascular tissue was evaluated by ELISA.
Results: Presence of tumor was resulted in significant inhibition of response to ACh in both 4TLM- and 4TBM-injected mice but not 67NR-injected mice. Furthermore, both KCl and Phe-induced contraction of thoracic aorta was not changed significantly in tumor-bearing animals. eNOS and p-eNOS expressions decreased while gp91phox and TNF-α expressions increased in endothelium significantly in mice with metastatic breast carcinoma compared with 67NR-injected and control mice. Moreover, TNF-α levels of thoracic aorta in 4TLM and 4TBM mice were higher than that of 67NR mice. Tumor-induced endothelial dysfunction determined by ACh-induced relaxation improved by superoxide dismutase (SOD), apocynin (a NADPH oxidase inhibitor), and infliximab (a TNF-α monoclonal antibody).
Conclusion: The findings of this study suggest that presence of metastatic breast carcinoma may cause a significant reduction in endothelium-dependent relaxation of thoracic aorta via NADPH oxidase-mediated oxidative stress and TNF-α production.
Disclosure of Interest: None declared.

PP117—THE VARYING EFFECTS OF PENTOXIFYLLINE ON CYCLIN D1 LEVELS AND G1 PHASE ARREST IN DIFFERENT RENAL CELL CARCINOMA CELL MODELS

N. Mastrandrea*; W.L. Cai; K.Y. Tham; T.J. Monks; and S.S. Lau
Pharmacology & Toxicology, University of Arizona, Tucson, Arizona
Introduction: Cyclin D1 is required for cells to progress from the G_1 phase of the cell cycle into the S phase. Mutation or overexpression of cyclin D1, often occurring in tumors, leads to uncontrolled cell cycle progression and proliferation. QTRRE (rodent) as well as ACHN and 786-O (human) cell models of renal cell carcinoma (RCC) display elevated cyclin D1 protein levels. Pentoxifylline (PTX), an FDA-approved competitive, nonspecific phosphodiesterase inhibitor, has found recent use as an adjunct in chemotherapy for patients to help treat cachexia and capillary leak syndrome.
Patients (or Materials) and Methods: We utilized Western blot analysis as well as propidium iodide-based cell cycle analysis to study the effects of PTX in our RCC cell models.
Results: Initially (0–8 hours), PTX induced a time- and dose- (35 μM–3.5 mM) dependent decrease in cyclin D1 levels in the RCC cell models. Cotreatment with PTX and the protein translation inhibitor, cycloheximide (5–25 μM), revealed a decrease in cyclin D1 protein half-life. Furthermore, the PTX-induced decrease of cyclin D1 was abolished in the presence of a proteasome inhibitor (MG-132, 10 μM) in all 3 RCC cell models. Subsequently (12–72 hours), PTX caused maximal cyclin D1 decreases in QTRRE and ACHN cells, whereas cyclin D1 levels in 786-O cells recovered and surpassed initial amounts seen in the control. Concomitant with cyclin D1 levels, QTRRE and ACHN cells demonstrated enhanced G1 phase cell cycle arrest at 24 hours (170% and 140%, respectively) compared with 786-O cells (107%) and the nontumorigenic human kidney cell line HK-2 (107%).
Conclusion: The data suggest that PTX decreases cyclin D1 protein levels by stimulating proteasomal degradation (and subsequent G_1 phase arrest), which is sustained in the QTRRE and ACHN but not 786-O RCC cell models. The data reveal a need to better understand the differences in PTX response between the RCC cell models. Moreover, an ability to predict a positive response to PTX may contribute to better personalized cancer therapy treatment for patients. Because our findings also reveal a novel anticancer chemotherapeutic property of PTX, the utility of PTX as an adjuvant therapy in the treatment of cancer should be further explored.
Disclosure of Interest: None declared.

PP118—IN VITRO STUDY OF SONODYNAMIC AND PHOTODYNAMIC TREATMENT ON HUMAN CANCER CELL LINES

F. Foglietta[1*]; R. Canaparo[1]; A. Francovich[2]; P. Civera[2]; and L. Serpe[1]
[1]Dipartimento di Scienza e Tecnologia del Farmaco, University of Torinoy; and [2]Dipartimento di Elettronica, Politecnico of Torino, Torino, Italy
Introduction: Photodynamic therapy (PDT) is an anticancer treatment that uses light to activate cytotoxic compounds killing cancer cells. Sonodynamic therapy (SDT) is a new anticancer treatment where ultrasound is used to trigger the cytotoxic effect of chemical compounds known as sonosensitizers. SDT is able to focus the ultrasound energy, generated by selected continuous or pulsed ultrasound such as shock waves (SW), onto malignant sites situated deeply inside tissues overcoming the main drawback linked to the use of PDT: the poor penetration of light in biological tissues. Even if the SDT mechanism is still under debate, some researchers suggest a common basic

principle for PDT and SDT. Therefore, in vitro comparison of PDT and SDT effects can provide insight into SDT mechanism of action. We have investigated PDT and SDT effects on human melanoma and fibrosarcoma cell lines, previously exposed to 5 aminolevulinic acid (Ala), through cell proliferation, cell death, and gene expression analysis.

Patients (or Materials) and Methods: The human melanoma, A2058, and fibrosarcoma, HT-1080, cell lines were previously exposed to Ala (0.45 mM) for 12 and 4 hours, respectively. SW generated by a piezo-electric device (Piezoson 100, Wolf) were used for SDT. In particular, A2058 were treated with an energy flux density (EFD) of 0.32 mJ/ mm_2 for 1000 shots (4 shots/sec) while HT-1080 were treated with an EFD of 0.43 mJ/mm_2 for 500 shots (4 shots/sec). A LED lamp at 405 nm was used for PDT and both cell lines were treated for 5 min at 15 mW. Cell growth was evaluated by WST-1 assay, cell death by flow cytometric analysis with SYTOX Green and APC-Annexin V and mRNA expression by real-time RT-PCR.

Results: In A2058 both treatments determined a significant cell growth reduction even if SDT produced a progressive cell growth decrease compared with PDT reaching the greatest decrease at 72 hours ($P < 0.01$). Moreover, cell death evaluation highlighted a 25% increase of apoptotic cells at 48 hours from SDT. Both PDT and SDT determined a significant overexpression of the pro-apoptotic gene *BAX* and of the genes involved in the oxidative stress, *NQO1 and SOD2*. In HT-1080, SDT was more effective than PDT with a more significant increase of apoptotic cells compared with PDT ($P < 0.01$).After both treatments a significant overexpression of the pro-apoptotic gene *APAF1* was observed.

Conclusion: After PDT and SDT a similar gene expression profile was observed in both cell lines, even though SDT seems more effective on fibrosarcoma cells and PDT on melanoma cells.

Disclosure of Interest: None declared.

PP119—EFFECT OF LIPOSOMAL CURCUMIN ON RED BLOOD CELLS IN VITRO

A. Storka[1*]; B. Vcelar[2]; L. Helson[3]; and M. Wolzt[1]
[1]*Clinical Pharmacology, Medical University of Vienna, Vienna;* [2]*Immunbiologische Forschung GmbH, Polymun Scientific, Klagenfurt, Austria; and* [3]*Inc., SignPath Pharma, Quakertown, United States*

Introduction: Curcumin possesses anticancer, anti-inflammatory, antioxidant, and antimicrobial properties. Curcumin has a poor oral bioavailability and solubility in plasma. Accordingly, various drug delivery systems, such as liposomal preparation, have been developed for intravenous administration. However, animal studies have shown dose-dependent hemolysis after infusion of liposomal curcumin. Because blood cells are the first point of contact for liposomal curcumin when administered intravenously, we investigated the influence of curcumin on human red blood cell (RBC) morphology in vitro.

Patients (or Materials) and Methods: Whole blood buffered with EDTA was incubated with different concentrations (1, 10, 100 μg/mL) of free or liposomal formulations of curcumin. RBC morphology and mean cellular volume were examined after 30 minutes, 1 hour, 2 hours, and 4 hours of incubation.

Results: Dose-dependent echinocyte formation was observed after incubation with free and liposomal Curcumin, with a threshold concentration of 10 μg/mL and peak effect after 30 minutes of incubation. Treatment with empty liposomes also resulted in RBC shape change. A concomitant increase in mean red blood cell volume was detectable.

Conclusion: Curcumin, liposomes and liposomal curcumin dose-dependently change RBC morphology. This effect is additive and may represent a first sign of toxicity following intravenous administration.

Disclosure of Interest: A. Storka: None declared. B. Vcelar: None declared. L. Helson: Other: Chief Executive Officer of SignPath Pharma. M. Wolzt: None declared.

PP120—CHEMOTHERAPEUTIC ANTITUMOR ACTIVITIES OF CURCUMIN

A.M. El-Medany[1*]; H.H. Hagar[2]; O.A. Nayel[1]; and J.M. El-Mednay[3]
[1]*Clinical Pharmacolgoy, Alexandria University College of Medicin, Alexandria;* [2]*Clinical Pharmacolgoy, Zagazig University College of Pharmacy, Zagazig; and* [3]*Anatomy, Alexandria University College of Medicin, Alexandria, Egypt*

Introduction: In search for drugs that can target cancer cell microenvironment in as much as being able to halt malignant cellular transformation, the natural dietary phytochemical curcumin was currently assessed in DMH-induced colorectal cancer rat model.

Patients (or Materials) and Methods: The study enrolled 50 animals divided into a control group (n = 10) and DMH-induced colorectal cancer control group (n = 20) (20 mg/kg.-body weight for 28 weeks) versus curcumin-treated group (n = 20) (160 mg/kg suspension daily oral for further 8 weeks).

Experimental Procedures: *ACF Assay:* The colonic lesion biopsies were stained by 30% methylene blue for light microscopic examination [using a 40 magnification to transluminate the specimens] in search for lesions fulfilling Mc Lellan and Bird criteria

Histopathological Examination: By using light microscopic assessment.

Biochemical Estimations in Colonic Tissues: Malondialdehyde (MDA) Assay, reduced glutathione (GSH) Assay, Cyclooygenase-2 (COX-2) activity measurement, tumor necrosis factor-α (TNF-α1 Determination, platelet activating factor (PAF) activity measurement, transformaing growth factor-β (TGF-β) determination*:*

Results: Treatment by curcumin succeeded to significantly decrease the percent of ACF and tended to normalize back the histologic changes retrieved in adenomatous and stromal cells induced by DMH. The drug also significantly elevated GSH and significantly reduced most of the accompanying biochemical elevations (namely MDA, TNF-α, TGF-β, and COX2) observed in colonic carcinomatous tissue, induced by DMH, thus succeeding to revert that of MDA, COX2& TGF-β back to near normal as justified by being nonsignificantly altered compared with normal controls. The only exception was PAF, which was insignificantly altered by the drug.

Conclusion: When taken together, it could be concluded that curcumin possess the potentiality to halt some of the orchestrated cross-talk between cancerous transformation and its microenvironmental niche that contributes to cancer initiation, progression, and metastasis in this experimental cancer colon model. Envisioning these merits to a drug with an already known safety preferentiality, awaits final results of current ongoing clinical trials, before curcumin can be added to the new therapeutic armamentarium of anticancer therapy.

Disclosure of Interest: None declared.

PP121—CYP2C9 GENOTYPES ASSOCIATED WITH HIGHER SULFOLANE LEVELS IN CHILDREN RECEIVING INTRAVENOUS BUSULFAN PRIOR TO HEMATOPOIETIC STEM CELL TRANSPLANTATION

C.R.S. Uppugunduri[1,2*]; Y. Daali[3]; M.A. Rezgui[4]; P. Huezo Diaz[1,2]; A.K. Tyagi[1,2]; J. Rousseau[4]; M. Duval[4,5]; H. Bittencourt[4,5]; M. Krajinovic[4,6,7]; and M. Ansari[1,2]
[1]*Department of Pediatrics, Hemato-oncology unit, University of Geneva;* [2]*CANSEARCH research laboratory, Geneva Medical*