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# **ADVANCES IN PROSTAGLANDIN AND LEUKOTRIENE RESEARCH**

**BASIC SCIENCE  
AND NEW CLINICAL APPLICATIONS**

edited by

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E. GRANSTRÖM AND S. NICOSIA

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# Advances in Prostaglandin and Leukotriene Research

Basic Science and New Clinical Applications

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## LIPID PEROXIDATION AND CYCLOOXYGENASE-2 ACTIVITY IN HUMAN HEPATOCELLULAR CARCINOMA CELL LINES WITH AND WITHOUT MULTIPLE DRUG RESISTANCE: CORRELATION WITH CELL GROWTH

Emanuela Masini, Ornella Fantappiè, Iacopo Sardi, Laura Raimondi, Valentina Fabbroni, Pier Francesco Mannaioni, and Roberto Mazzanti

### Introduction

Intrinsic or acquired resistance to antineoplastic drugs is the main cause of failure in cancer chemotherapy. Human hepatocellular carcinoma (HCC) has been increasing on a worldwide scale and it is among the most chemoresistant tumors. One of the main mechanisms of drug resistance is related to the over-expression of the gene that encodes a transmembrane *P*-glycoprotein which is involved in drug extrusion from the cells that possess the multiple drug-resistant (MDR1) phenotype [1]. Cancers with an MDR1 phenotype are more resistant to several anticancer drugs. Free radical formation is purported to be involved in cytotoxic effects of anticancer agents. However in HCC cells the expression of MDR1 phenotype *per se* does not increase significantly the resistance to iron-stimulated lipid peroxidation [2]. Moreover hepatitis B and C viruses are found to be important factors of hepatocarcinogenesis, through the induction of cell proliferation and angiogenesis. Cyclooxygenase-2 (COX-2) has now been identified as expressed in a number of cancers, including HCC [3], but whether COX-2 activity affects cell growth and susceptibility to free radical attack in human HCC cell lines has not been elucidated. To assess these points, an HCC MDR1 cell line highly resistant to doxorubicin, P1(0.5), and the parental drug-sensitive cells, P5, were studied for their susceptibility to lipid peroxidation and for the effect afforded by nimesulide, a COX-2 inhibitor.

### Methods

Experiments were performed on a HCC cell line (PLC/PRF/5). The P1(0.5) MDR clone, selected and maintained in 0.5 µg/ml doxorubicin [2] and the parental drug-sensitive cells (P5) were used. Cell lines were cultured in DMEM supplemented with 10% fetal calf serum (FCS), 100 U of penicillin, 10 µg/ml streptomycin, and 0.25 µg/ml amphotericin B, at 37°C in 5% CO<sub>2</sub>. Susceptibility to the free radical attack was assessed by measuring lipid peroxidation by the determination of thiobarbituric acid-reactive substances (TBARS) in the reaction mixture after 60 minutes of incubation at 37°C of cells exposed to ascorbate-Fe

(0.5 mM and 25  $\mu$ M FeSO<sub>4</sub>) [4]. Alphatocopherol (vitamin E) content in the cells was measured using a HPLC method [2]. PgE<sub>2</sub> production was determined in the supernatants by a RIA method. P5 and P1(0.5) cell growth was evaluated by [<sup>3</sup>H]thymidine incorporation assay. Briefly, 10<sup>4</sup> cells were seeded in 24-well plates and left to grow to subconfluence. After 24-hour incubation in a steady-state medium (0.1% FCS), the medium was substituted with DMEM containing 10% FCS, added with epidermal growth factor (EGF, 100 nM) in the presence or absence of nimesulide, a COX-2 inhibitor, at the noted concentrations. At the end of the incubations, the cells were pulsed for 4 hours with 0.5  $\mu$ Ci per well of [*methyl*-<sup>3</sup>H]thymidine (specific activity, 46 Ci/mmol). The medium was then removed, and DNA was precipitated with 3% trichloroacetic acid (TCA) and extracted with 1 ml of 0.3 M NaOH. The recovered radioactivity was measured in a beta scintillation counter. The experiments were performed in quadruplicate, and the values were expressed as dpm/well (mean+SE). Statistical analysis was performed using a multifactor ANOVA (Scheffe's *t* test) and Student's *t* test for unpaired values. *P* < 0.05 was considered significant.

### Results and Discussion

Concentrations of TBARS measured at time 0 was similar in P5 and P1(0.5) cells. Exposure to ascorbate-Fe of P5 cells significantly increased TBARS production, which was left unchanged in ascorbate-Fe treated P1(0.5) cells (Figure 1a). Vitamin E cell content was significantly higher (*p* < 0.05) in P1(0.5) cells expressing the MDR phenotype (Figure 1b).

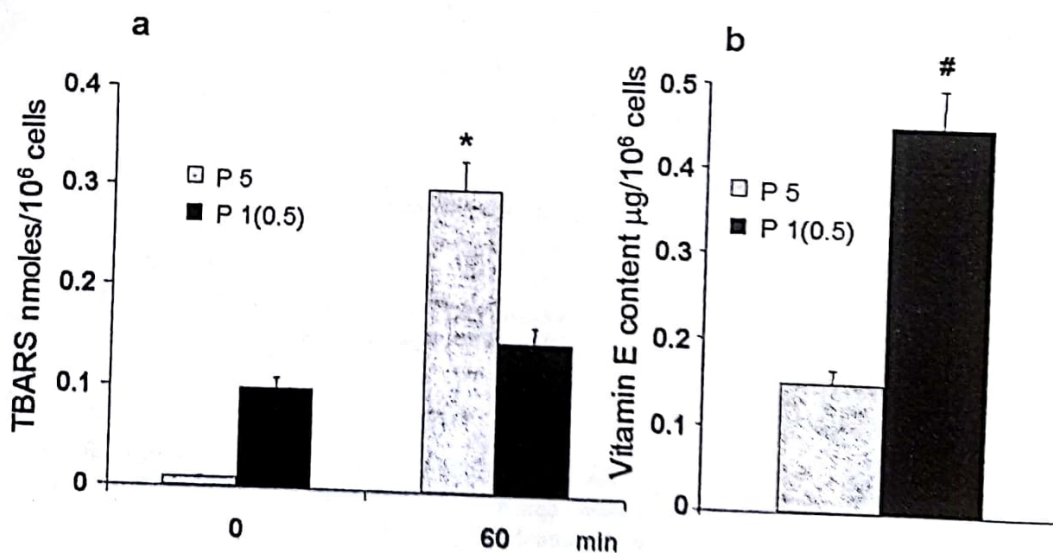


Figure 1. TBARS concentration at time 0 and after 60 minutes exposure to ascorbate-Fe (panel a), and vitamin E content (panel b). \* *p* < 0.05 versus 0 minutes.; # *p* < 0.05 versus P5.

Basal  $\text{PgE}_2$  production was higher in P1(0.5) cells than in P5 cells. The exposure to 100 nM EGF increased  $\text{PgE}_2$  production in both cell clones but the amounts of  $\text{PgE}_2$  produced were significantly higher in P1(0.5) cells. The treatment with nimesulide (10  $\mu\text{M}$ ), a COX-2 inhibitor, blunted the EGF-induced  $\text{PgE}_2$  production in both cell clones (data not shown). The involvement of COX-2 in cell growth is shown by the effect of nimesulide on [ $^3\text{H}$ ]thymidine incorporation induced by EGF: nimesulide significantly reduced EGF-stimulated thymidine incorporation in P1(0.5) cell clone but not in P5 one (Figure 2).

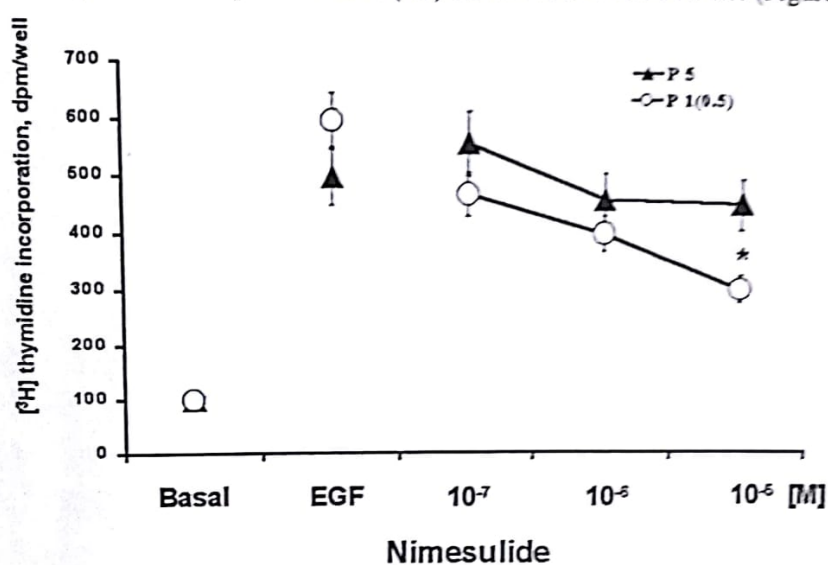


Figure 2. Effect of nimesulide on EGF(100 ng/ml)-stimulated [ $^3\text{H}$ ]thymidine incorporation in P5 and P1(0.5) cell clones. \*  $p < 0.01$ .

These data suggest that the resistance of HCC to undergo lipid peroxidation is related to an increased vitamin E content rather than to MDR1 phenotype; while the MDR1 phenotype is associated with a COX-2 up-regulation and COX-2 control of mitogen-activated cell proliferation. In conclusion COX-2 inhibitors could have a role in the therapy of MDR HCC, by increasing the responsiveness of the cells to chemotherapy.

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