

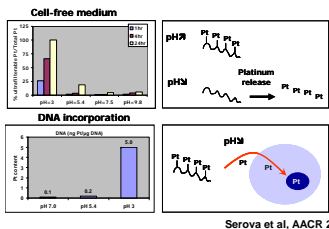
Role of p21 in sensitivity to DACH-platinum compounds, oxaliplatin and ProLindac™, in human cancer cells

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INTRODUCTION

- The platinum-based chemotherapeutic agents (cisplatin, carboplatin and oxaliplatin) exert their cytotoxic effects by binding with DNA in cancer cells, causing distortion and/or strand breaks, and consequently apoptotic cell death. A number of mechanisms are involved in the *in vitro* resistance to platinum agents such as decrease of intracellular accumulation and/or enhancing of DNA repair.
- ProLindac™ is a novel formulation of a DACH-platinum compound comprising a hydrophilic biocompatible copolymer that acts as a macromolecular carrier, and a bioactive DACH-platinum complex linked by means of a tri-glycine spacer group.
- The polymer delivery system aims to reduce the damaging effects of DACH-platinum in healthy tissues, which can markedly improve the treatment safety profile.



- It was recently shown that exposure to oxaliplatin in colon cancer cells induced specific significant changes in the expression of many genes implicated in drug transport, DNA repair and cell cycle regulation (Voland C et al., Mol Cancer Ther 2006). In this study we compared the genetic effects induced by ProLindac™ with those induced by oxaliplatin and cisplatin in cancer cells.

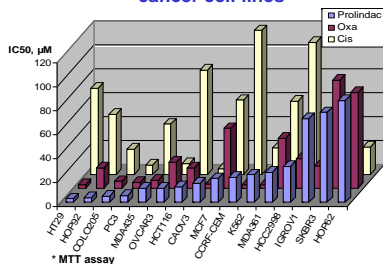
AIM OF THE STUDY

- To evaluate the effects of ProLindac™ on the expression of several cell cycle and DNA repair-related genes aiming to identify biomarkers for ProLindac™ sensitivity.

MATERIALS AND METHODS

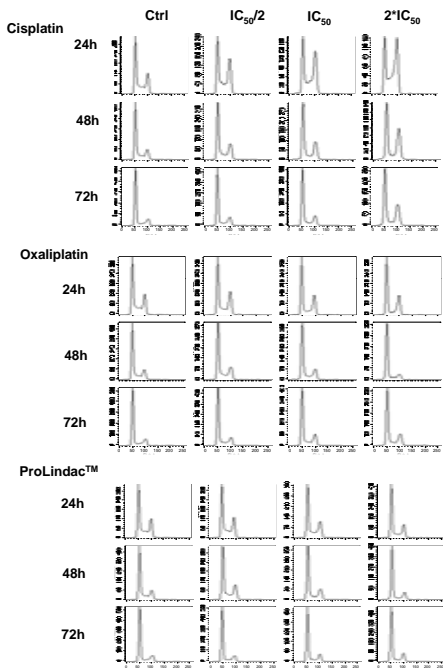
- The *in vitro* activities of cisplatin, oxaliplatin and ProLindac™ were evaluated in a panel of 16 cancer cell lines previously characterized for expression of genes implicated in drug resistance, and drug transport and metabolism (Serova et al., AACR 2008). Two isogenic colon cancer cell lines were also studied: parental HCT116 (p21+/+) cells and a HCT116 subline (p21-/-) in which the p21 gene has been deleted by targeted homologous recombination.
- MTT assay: cells seeded at 2x10³/well in 96-well plates and treated with different concentrations of drugs for various times, were incubated with 0.4 mg/ml MTT for 4 hours at 37°C. After incubation, the supernatant was discarded, the cell pellet was resuspended in 0.1 ml DMSO and the absorbance was measured at 560 nm. Growth inhibition curves were plotted as a percentage of untreated control cells.
- Cell cycle and apoptosis were studied by FACS analysis.
- Expression of selected genes was determined using quantitative-RT-PCR. Total RNA was extracted from 3 cell lines (parental HCT116 and 2 sublines with overexpression of MMR genes and deletion of p53), reverse-transcribed and analyzed by RT-PCR (TaqMan instruments). Relative mRNA level was calculated using TATA-binding protein (TBP) reference gene expression.

Cytotoxicity of ProLindac™, oxaliplatin and cisplatin given for 72 h in a panel of 16 cancer cell lines



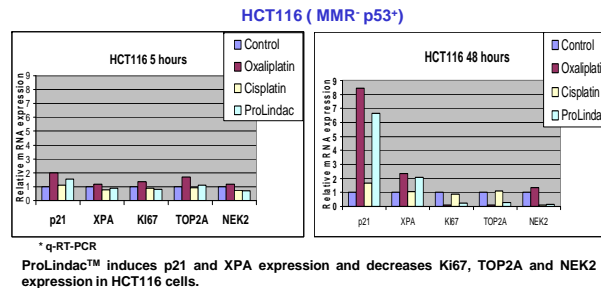
ProLindac™ demonstrated a cytotoxicity profile similar to that of oxaliplatin in our panel of cancer cell lines.

Effects of ProLindac™, oxaliplatin and cisplatin on the cell cycle in parent HCT116 cells over different exposure times

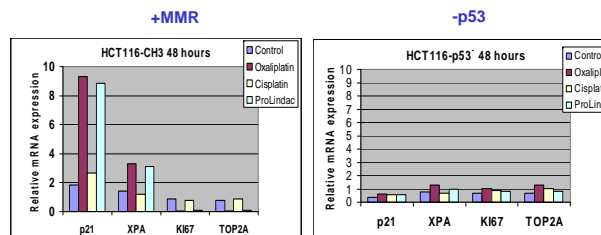


RESULTS

Effects of ProLindac™ on gene expression

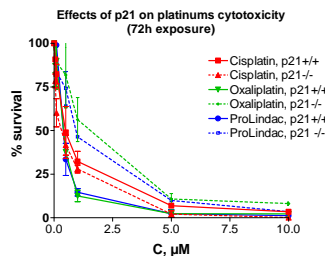


ProLindac™ induces p21 and XPA expression and decreases Ki67, TOP2A and NEK2 expression in HCT116 cells.



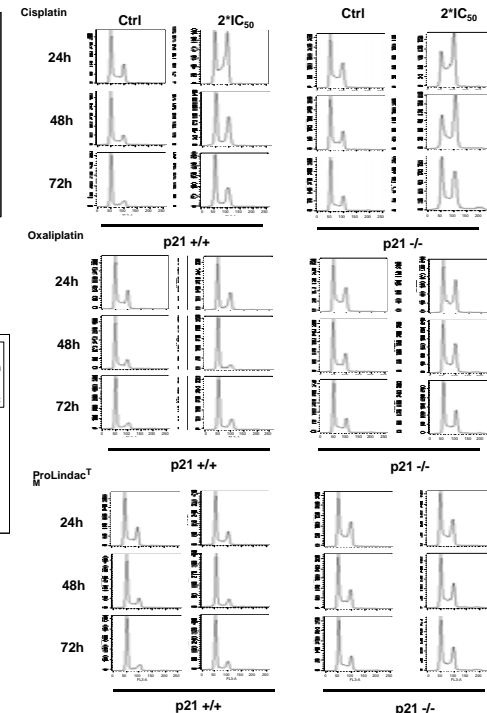
Effects of ProLindac™ and oxaliplatin on gene expression were MMR-independent and p53-dependent.

Effects of p21 expression on platinum cytotoxicity



Cells	IC50, µM (72h exposure)		
	Cisplatin	Oxaliplatin	ProLindac™
HCT116 p21 +/+	0.5±0.15	0.5±0.1	0.4±0.1
HCT116 p21 -/-	0.3±0.1	1.6±0.5	1.0±0.2

Effects of p21 expression on cell cycle changes after exposure to platinum



Cisplatin caused a G2M block, which was slightly less in HCT-116 p21+/+ than HCT-116 p21-/- cells. The G2M block was repaired in p21+/+ cells at 48 h treatment, while the p21-/- cells were still blocked at 72 h treatment.

In HCT-116 p21+/+ cells, oxaliplatin induced a slight increase in the G1 cells fraction during the first 24 h of treatment. Cell cycle perturbations were repaired subsequently. In p21-/- cells, oxaliplatin induced a slight increase in the G2M fraction.

The cell cycle perturbations induced by ProLindac™ (G2M block) were similar to those caused by oxaliplatin.

CONCLUSIONS

- ProLindac™ demonstrated cytotoxicity superior to cisplatin and similar to oxaliplatin in a panel of human cancer cell lines.
- Antiproliferative effects of ProLindac™ were associated with increased expression of p21 and XPA in p53+ but not in p53- cells
- ProLindac™ was more active in p21+ than in p21- cells. Oxaliplatin, but not cisplatin, also displayed increased cytotoxicity against p21+ cells.
- Our findings confirm the DACH-platinum mechanistic characteristics of ProLindac™ and suggest a role for p21 as a potential biomarker of DACH activity.