mismatch primers and restriction enzyme *BstXI*. All of PCR-RFLP results were confirmed by direct sequencing. The sensitivity and specificity of PCR-RFLP in the detection of *K-ras* mutation was confirmed in cancer cells with wild-type (H1703) and mutant (A549) *K-ras* genes and in a serial dilution assay.

Results: The results confirmed that PCR-RFLP was able to detect a mutation in a sample containing a mixture of mutant and wild-type DNA in a 1:10 ratio. Then, using the serum DNA of 12 colon cancer patients with *K-ras* mutation detected in tissue DNA, we were able to confirm the presence of *K-ras* mutation in serum DNA from 3 of 5 patients with known *K-ras* mutation.

Conclusion: The PCR-RFLP assay is a useful tool for detecting *K-ras* mutations from serum DNA and allows early diagnosis of molecular determinants in colon cancer patients.

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ABSTR 0932 – Poster Presentation

Cytotoxicity and Pharmacokinetics of Four Platinum Salts in Human Colon Carcinoma Cell-Line HCT116

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Background: Adding oxaliplatin to 5-FU–based regimens improves outcomes of patients with colorectal cancer in the metastatic and adjuvant settings. The benefit of adding oxaliplatin (or other radiosensitizers) to chemoradiotherapy for rectal cancer has been suggested, but the best oxaliplatin schedule is yet to be determined. Newer liposomal formulations of platinums have been proposed to allow higher intracellular concentrations of platinum with limited toxicity. Understanding the cytotoxic mechanisms of platinum-based drugs and elucidating their underlying pharmacokinetics are crucial to improve their efficiency as radiosensitizers, and to determine the best treatment scheme for these patients. We studied the cytotoxic effects on human colorectal cancer cell line, the intracellular accumulation, and the DNA binding for Lipoplatin™ and Lipoxal™, the liposomal formulations of cisplatin and oxaliplatin, respectively, which were compared to the liposome-free platinum compounds.

Methods: The human colorectal cancer cell-line HCT116 cells was used. Cell growth inhibition by platinum derivatives was evaluated with a colony formation assay. The inhibitory concentration (IC_{50}) for each drug was determined. Cells exposed to cisplatin, oxaliplatin, Lipoplatin™ and Lipoxal™ at the IC_{50} concentration were analyzed for their intracellular accumulation and DNA-binding of platinum using inductively coupled plasma mass spectrometry at 1, 4, 8, 24, and 48 h from exposure.

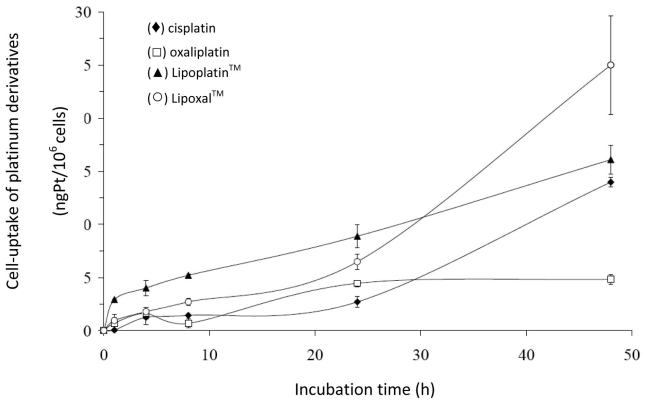

Figure 1: Time course of the cellular accumulation of platinum derivatives in HCT116 cells. Cells were incubated at the IC_{50} concentration previously measured after 4 h incubation. The amount of platinum accumulated in the cells was measured using ICP-MS. Each point represents the mean ± SD (n=3).

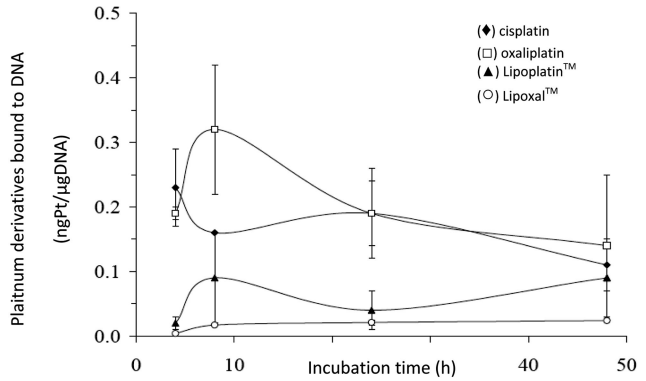

Figure 2: Time course of the binding of platinum to DNA after exposing the HCT116 cells. Cells were incubated at the IC_{50} concentration previously measured after 4 h incubation. The amount of platinum accumulated in the cells was measured using ICP-MS. Each point represents the mean ± SD (n=3).

Results: The colony formation assays showed an IC_{50} of 7, 7.5, 21, and 70μM, for oxaliplatin, cisplatin, Lipoxal™, and Lipoplatin™, respectively. The liposomal formulations had reduced cytotoxicity on the HCT116 cells. The cellular uptake for three platinum derivatives continued to increase with time, except for oxaliplatin, which reached a plateau after 24 h incubation. Despite a higher intracellular accumulation, liposomal oxaliplatin provided lower DNA-bound platinum than the regular formulation. These data suggest that the liposomal oxaliplatin accumulated in the cancer cell might be relatively stable, which prevents the release of free oxaliplatin, impeding its binding to DNA.

Conclusion: Our results support that incorporation of cisplatin and oxaliplatin in a liposomal formulation can reduce their cytotoxicity *in vitro*. Despite higher intracellular concentration, a smaller fraction is incorporated into DNA. Our subsequent trials on combined chemoradiotherapy will determine if the DNA-bound platinum will reflect the radiosensitizing effect for each drug.