Kinase Inhibitors AZD5438 and Dabrafenib Alleviate Cisplatin-Induced Acute Kidney Injury and Hearing Loss

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Purpose of the study

Cisplatin is an effective chemotherapy agent for a wide variety of solid tumors, but its beneficial use is dose-limited by serious side effects including acute kidney injury (AKI) and hearing loss. There are currently no FDA-approved drugs to treat AKI and only one drug was approved to treat hearing loss in pediatric localized hepatoblastoma. Recently, two oral drugs, kinase inhibitor AZD5438, a Phase-2 clinical trial CDK2 inhibitor, and dabrafenib, an FDA-approved BRAF kinase inhibitor, were identified as protective against cisplatin-induced hearing loss in mice. Here, we hypothesized that similar cell stress and death pathways are activated in kidney and inner ear cells when exposed to cisplatin and tested if these drugs can also alleviate cisplatin-induced AKI.

Methods

AZD5438 and dabrafenib protective effects against cisplatin-induced cell death were tested initially in a human kidney tubular cells HK-2. Next, we examined the two drugs in an established AKI FVB adult mouse model, giving orally AZD5438, 35 mg/kg, twice a day, and dabrafenib, 12 mg/kg, three times a day, for three consecutive days, to cisplatin-treated (25 mg/kg) mice. Protection of nephrotoxicity was evaluated by reduced levels of serum acute kidney injury markers- blood urea nitrogen (BUN), creatinine, renal neutrophil gelatinase-associated lipocalin (NGAL), histology markers, reduced levels of biomarkers PCNA and p-ERK, and suppression of cell death by TUNEL assay. In addition, we studied the CDK2 KO genetic mouse model as a system for CDK2 inhibition in cisplatin-induced AKI.

Results

AZD5438 & Dabrafenib Protect Human Proximal Tubule Cell Line (HK-2 Cells) From Cisplatin-Induced Cell Death And Do Not Affect Tumor Killing Efficacy Of Cisplatin

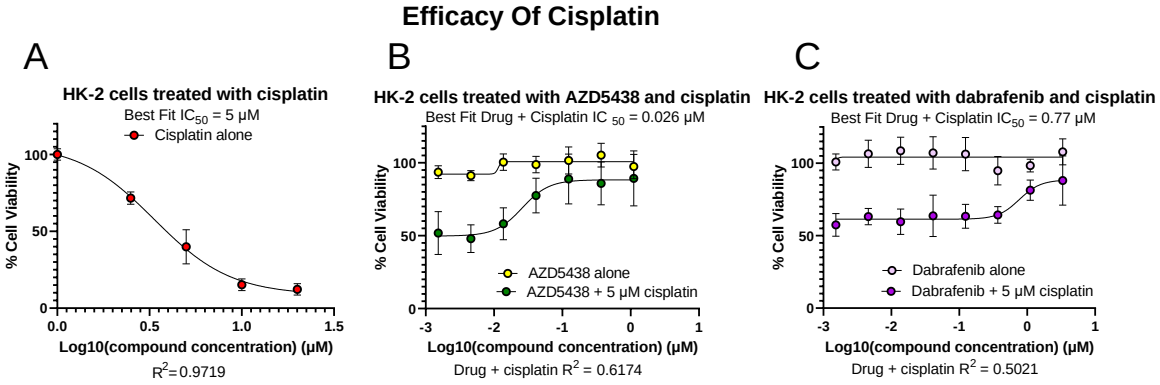

Figure 1: **AZD5438 & dabrafenib protect proximal tubule epithelial cell line (HK-2 cells) from cisplatin-induced cell death.** (**A**) HK-2 cells were treated with increasing concentrations of cisplatin for 48 hours. Cell viability assay was done, and dose-response curve demonstrates dose dependent increase in cell death of HK-2 cells and shows an IC_{50} of 5 µM. **(B & C)** HK-2 cells were pretreated for 1 hour with (**B**) AZD5438 or (**C**) dabrafenib and co-treated with 5µM cisplatin and AZD5438 or dabrafenib for 48 hours. Dose-response curve showed protection from cisplatin-induced cell death.

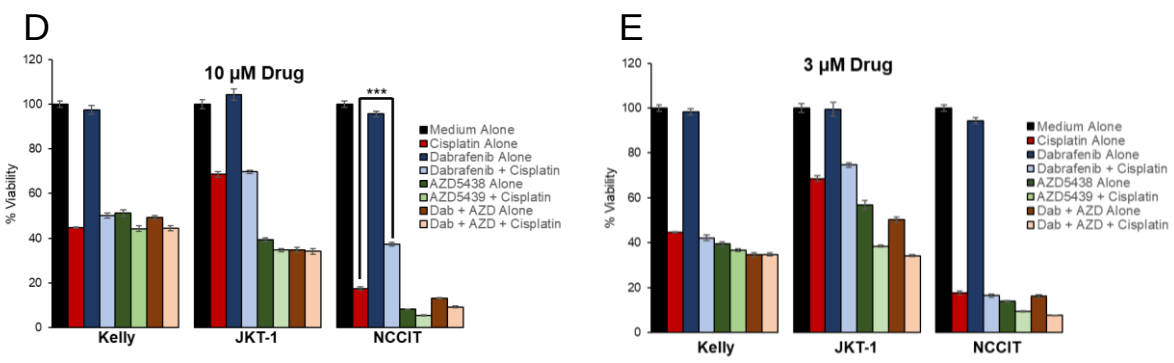

Figure 2: AZD5438 & dabrafenib do not affect tumor killing efficacy of cisplatin (**D & E**) **Tumor cell lines (neuroblastoma and testicular cancer cell lines)** were treated with drugs and cisplatin for 48 hours. Cell viability assay was done, and dose-response curve demonstrates that cisplatin-induced tumor cell death was not affected by co-treatment with AZD5438 or Dabrafenib.

Results

AZD5438 & Dabrafenib Mitigate Cisplatin-Induced Kidney Injury In Adult FVB Mice

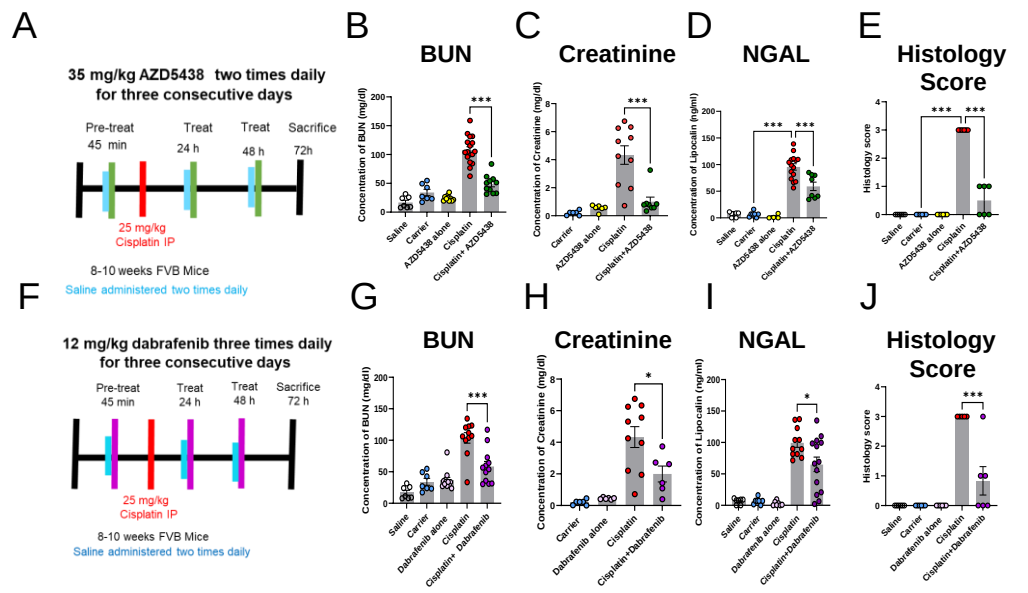

Figure 3: AZD5438 & dabrafenib protect against cisplatin-induced kidney injury in adult FVB mice. **(A&F)** Experimental design of cisplatin injection and AZD5438 & dabrafenib administration. **(B-D; G-I)** Adult FVB mice injected with cisplatin and cotreated with AZD5438 or dabrafenib were analyzed for serum kidney injury biomarker BUN, Creatinine & NGAL **(E &J)** Renal tissues were stained with H&E and PAS stain. Kidney histological scores from H&E and PAS staining were obtained by pathologist blinded to the experimental conditions using a semi-quantitative pathological scoring system. Scores are expressed as mean ± SEM. Scoring system based on percentage of damage -normal-0,25%-1+, 25%-50%-2+, 50%-75%-3+, 75-100%-4+. Numbers inside each bar denote number of mice used per treatment. Values expressed are means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared by one-way ANOVA with Bonferroni post hoc test.

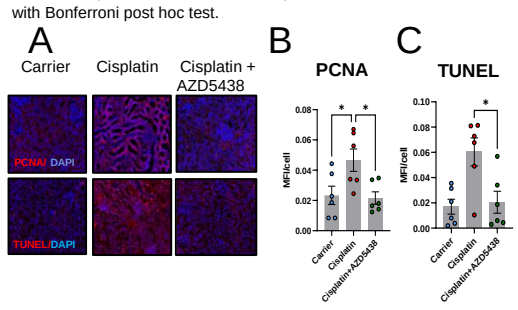

Figure 4: AZD5438 downregulates tissue damage and PCNA expression on day 3 after cisplatin injection. Representative images (**A**) of Immunostaining shows downregulation of (**B**) PCNA and (**C**) cell death in mice treated with AZD5438.

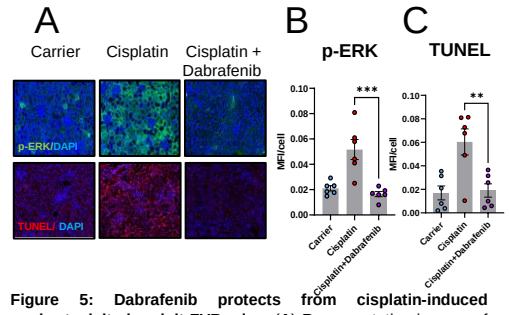

Figure 5: Dabrafenib protects from cisplatin-induced nephrotoxicity in adult FVB mice. (**A**) Representative images of immunostaining. Reduced levels of (**B**) pERK protein and (**C**) cell death with cisplatin and dabrafenib compared to cisplatin alone after 72 hours of treatment.

CDK2 KO Mice Are Resistant To Cisplatin-Induced Kidney Injury Compared To WT Littermates

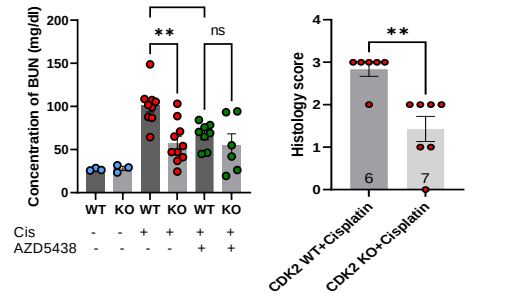

Figure 6: CDK2 KO mice showed significant resistance to cisplatin induced kidney damage. CDK2 WT & KO, FVB background mice were treated with cisplatin and sacrificed after 72 hours (**A**) Significant decrease in BUN Levels (**B**) Significant decrease in tissue damage shown by histology. Numbers inside each bar denote number of mice used in treatment. Means ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 compared to cisplatin-alone by T- test and one-way ANOVA with Bonferroni post hoc test.

AZD5438 and Dabrafenib Enhance Survival Rate of Cisplatin Treated Mice

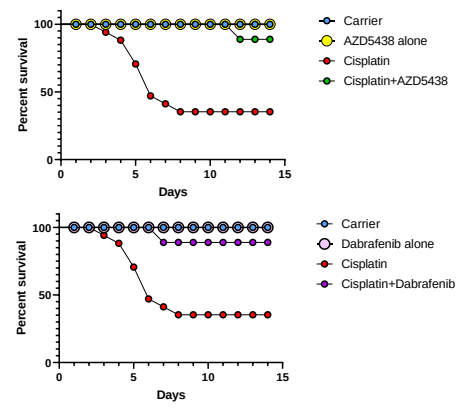

Figure 7: AZD5438 and dabrafenib enhanced survival rates of cisplatin injected mice. 8-week-old FVB mice were injected with cisplatin and treated with AZD5438 (35 mg/kg) and dabrafenib (12mg/kg) for 3 days. The survival percentage of the cisplatin mice with (green or violet) and without (red) treatment with AZD5428 and dabrafenib is shown.

Conclusions

The drugs reduced cisplatin-induced cell death in the HK-2 cell line, and attenuated cisplatin-induced AKI in mice when administered at doses that were in the range of those approved for human use. In summary, we show that similar cellular mechanisms can contribute to damage from cisplatin in the inner ear and kidney tissues, highlighting AZD5438 and dabrafenib as promising potential therapeutic candidates to treat cisplatin-induced kidney damage and hearing loss.

Acknowledgement

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