Novel platinum-based chemotherapeutic agents halt cholangiocarcinoma progression through the induction of inter-strand DNA breaks, preventing DNA repair mechanisms

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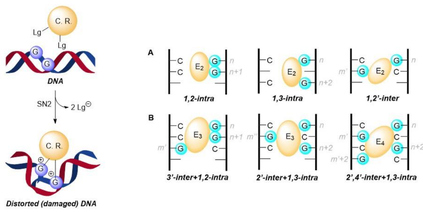
BACKGROUND

- **Cholangiocarcinoma (CCA)** comprises a heterogeneous group of malignant tumors with dismal prognosis.
- Its incidence is increasing worldwide, becoming a significant health problem.
- The first-line treatment for advanced CCA [cisplatin (CisPt) and gemcitabine] is considered palliative due to the high **chemoresistance** of this cancer.
- Platinum (Pt) derivatives are the most widely used chemotherapeutic agents for the treatment of malignant tumors.
- Cisplatin (CisPt) binds to the DNA causing mainly single-strand DNA breaks and consequent cancer cell death.

AIM

Design, synthesize and study a new generation of platinum (Pt)-derived chemotherapeutic drugs (Auriki-Pt) that produce higher ratios of inter-strand DNA breaks (*vs* more abundant single-strand breaks induced by CisPt and related compounds) and thus **hamper the development of DNA repair mechanisms in cancer cells.**

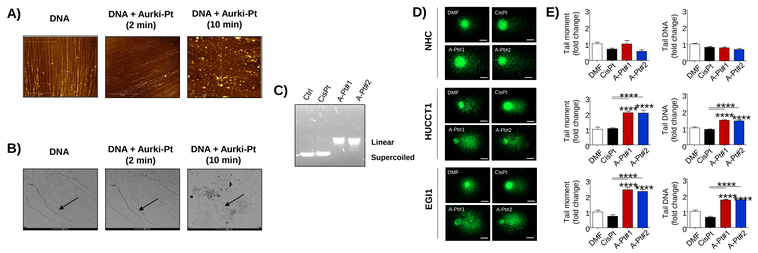


METHOD

- **Evaluation of the effect of Auriki-Pt on isolated DNA** from *Escherichia coli* using Atomic Force Microscopy **(AFM)** and Transmission Electron Microscopy **(TEM)**.
- **Evaluation of the effect of Auriki-Pt on DNA damage** using comet assay.
- **Evaluation of the antitumor effect of Auriki-Pt** on the viability, proliferation, spheroid formation and survival of human CCA cells (EGI1 and HUCCT1), newly generated CisPt-resistant CCA cells (EGI1R), normal human cholangiocytes (NHC) and cancer-associated fibroblasts (CAFs) ***in vitro***.
- **Evaluation of Auriki-Pt uptake** using indirect competition studies of known fluorescent substrate using flow cytometry and direct accumulation studies using HPLC-MS/MS.
- **Evaluation of the effect of Auriki-Pt** in a subcutaneous **mouse model** of CCA.

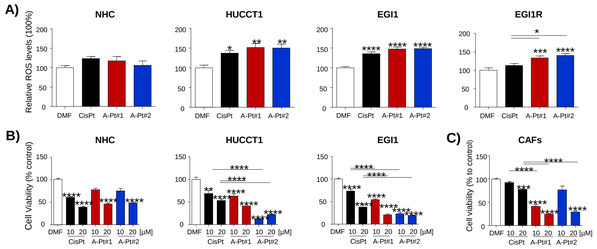
RESULTS

Figure 1: Auriki-Pt induces DNA damage on isolated DNA from *Escherichia coli* and on CCA cells *in vitro*



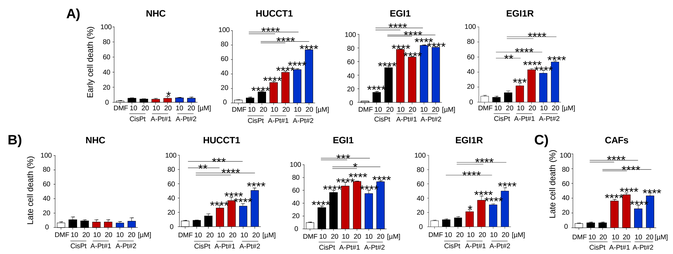
(A) AFM and **(B)** TEM images of isolated DNA from *Escherichia coli* untreated or after 10 minutes of incubation with Auriki-Pt. **(C)** Agarose gel electrophoretic mobility of pUC18 plasmid DNA when incubated with CisPt or Auriki-Pts. **(D)** Representative images of comet assays in NHC and CCA cell lines and **(E)** quantification of the DNA damage in NHC and CCA cell lines after CisPt or Auriki-Pt incubation at 10 μM for 48 hours. One-way ANOVA test was used. Data are shown as mean ± SEM.

Figure 2: Auriki-Pt#1 and Auriki-Pt#2 specifically reduce CCA cell viability and CAFs viability *in vitro*



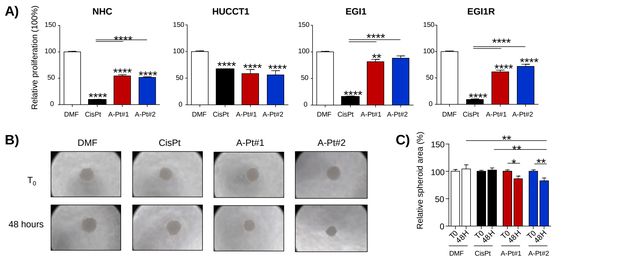
(A) Reactive oxygen species (ROS) formation in NHC and CCA cell lines after incubation with CisPt or Auriki-Pts for 48 hours. **(B)** Cell viability in NHC and CCA cell lines after incubation with CisPt or Auriki-Pts for 48 hours. **(C)** Cell viability in cancer-associated fibroblasts (CAFs) after incubation with CisPt or Auriki-Pts at for 48 hours One-way ANOVA test was used. Data are shown as mean ± SEM.

Figure 3: Auriki-Pt#1 and Auriki-Pt#2 specifically induce early and late cell death in CCA cell lines and in CAFs *in vitro*



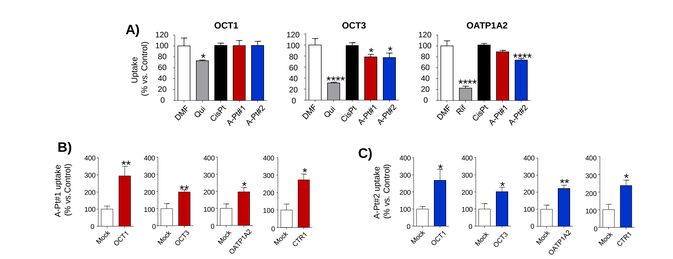
(A) Early cell death (Caspase-3) and **(B)** late cell death (Annexin-V/TO-PRO-3) of NHC and CCA cell lines (HUCCT1, EGI1, EGI1R) after CisPt or Auriki-Pt incubation for 48 hours by flow cytometry. **(C)** Late cell death (Annexin-V/TO-PRO-3) of cancer-associated fibroblasts (CAFs) after CisPt or Auriki-Pt incubation for 48 hours by flow cytometry. One-way ANOVA test was used. Data are shown as mean ± SEM.

Figure 4: Auriki-Pt#1 and Auriki-Pt#2 reduce CCA cell proliferation and induce spheroids shrinkage *in vitro*



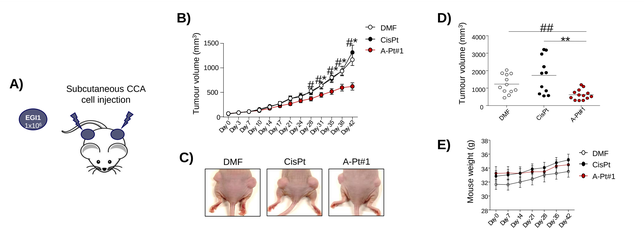
(A) Flow cytometry-based cell proliferation in NHC and CCA cell lines (HUCCT1, EGI1, EGI1R) after incubation with CisPt or Auriki-Pt at 10 μM for 48 hours. **(B)** Representative images of 3D spheroids of EGI1 CCA cells at baseline (T_0) and after incubation with Auriki-Pt and **(C)** quantification. One-way ANOVA test was used. Data are shown as mean ± SEM.

Figure 5: Auriki-Pt#1 and Auriki-Pt#2 uptake is mediated by OCT1, OCT3, OATP1A2 and CTR1, which do not transport CisPt



(A) Indirect competition assay in the presence or absence of the typical inhibitor of each transporter or Auriki-Pts 10 μM (Quinine for OCT1 and OCT3; Rifampicin for OATP1A2). Substrate content was determined by flow cytometry. Bar graphs representing **(B)** Auriki-Pt#1 and **(C)** Auriki-Pt#2 uptake in HepG2 or CHO mock cells or overexpressing OCT1, or OCT3 / OATP1A2 / CTR1, respectively determined by HPLC-MS/MS. Data are shown as mean ± SEM.

Figure 6: Auriki-Pt#1 halts CCA growth in a subcutaneous model of CCA



(A) Representative scheme of a subcutaneous model of CCA. **(B)** Tumor growth of CCA cells injected subcutaneously into immunodeficient mice in the presence of intraperitoneal administration of 0.5 mg/kg Auriki-Pt#1, CisPt or vehicle (DMF). **(C)** Representative images of tumors and **(D)** tumor volume of xenografts after sacrifice. **(E)** Mouse weight during treatment administration. One-way ANOVA and Two-way ANOVA tests were used. Data are shown as mean ± SEM. (# p<0.05 compared to DMF, * p<0.05 compared to CisPt)

CONCLUSIONS

- Auriki-Pts **selectively diminish CCA cell viability.**
- **Auriki-Pts induce higher DNA damage** in CCA cells than CisPt, thus being more effective triggering **apoptosis *in vitro***.
- Auriki-Pts **induce cell death in CisPt resistant** CCA cells.
- Auriki-Pts **reduce CCA cell proliferation** and induce **spheroid shrinkage.**
- Auriki-Pts **reduce CAFs viability** and induce **CAFs cell death**.
- The **uptake** of Auriki-Pts is mediated by **OCT1, OCT3, OATP1A2** and **CTR1,** which do not transport CisPt.
- **Auriki-Pt#1, but not CisPt, halts CCA tumor growth *in vivo***.
- Auriki-Pts represent a **promising therapeutic tool** for naïve or CisPt-resistant CCA tumors.

ACKNOWLEDGEMENTS

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