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Combined Effect of Gemcitabine (GEM) and SapC-DOPS Nanovesicles on Pancreatic Ductal Adenocarcinoma (PDAC) in Mice.

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Background: Phosphatidylserine (PS) is an anionic phospholipid primarily localized on the inner plasma membrane of healthy cells. In contrast, PS is externally expressed on cancer cells and tumor-associated vascular cells. PS is further externalized by treatment with GEM, a drug commonly used to treat advanced PDAC, the fourth leading cause of cancer-related death. The efficacy of GEM in combination with PS-targeted cancer-selective protein-lipid nanovesicles (SapC-DOPS) was studied *in vitro* and in PDAC mouse models.

Methods: MiaPaCa-2 subcutaneous PDAC tumors (100-300 mm³) were established in female athymic nude mice. Mice (12/group) were treated with GEM (40 mg/kg ip), SapC-DOPS (4.9 mg/kg iv), or both (GEM+SapC-DOPS) on day 26 and every 3 days thereafter. Tumor surface PS and size were analyzed at day 55. Orthotopic p53 2.1.1 tumors were generated in FVB/n mice and were confirmed by bioluminescence. 6 mice/group were treated with DOPS (1.9 mg/kg iv), GEM (10 mg/kg ip), SapC-DOPS (3 and 1.9 mg/kg iv), or GEM+SapC-DOPS 2x/week to day 55 and followed for survival. PS levels and cell death *in vitro* were confirmed by flow cytometry and MTT assay, respectively at 24-48 hr after GEM (10 nM-1 μ M) in human AsPC-1, MiaPaCa-2, murine p53 2.1.1 PDAC, and immortalized human pancreatic duct epithelial cells.

Results: Elevated PS surface expression on viable PDAC cells was confirmed 48 hr after GEM *in vitro* and in subcutaneous tumors. Cancer cell death was notably higher in GEM+SapC-DOPS treated mice compared to GEM or SapC-DOPS alone (75.5 \pm 2.1% vs. 25.0 \pm 1.5% and 17.3 \pm 5.1%, respectively). SapC-DOPS+GEM more effectively inhibited tumor growth in both subcutaneous and orthotopic xenograft models compared to GEM alone. SapC-DOPS+GEM improved survival vs. either drug alone.

Conclusions: The combination of GEM and SapC-DOPS demonstrated enhanced antitumor activity in PDAC. This suggests that targeting externalized PS combined with chemotherapy is an effective means of selectively inducing tumor cell death, providing support for clinical study.

Title:

Combined Effect of Gemcitabine (GEM) and SapC-DOPS Nanovesicles on Pancreatic Ductal Adenocarcinoma (PDAC) in Mice.

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