Namodenoson Inhibits the Growth of Pancreatic Carcinoma via De-regulation of the Wnt/β-catenin Signaling Pathway

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BACKGROUND
Pancreatic cancer is a very aggressive malignancy with an overall 5-year survival rate of 8%; in 52% of cases, the disease is diagnosed at stage IV.
- Systemic treatments for pancreatic cancer are lacking. The effect of gemcitabine alone, or in combination with other chemotherapies is modest, and checkpoint inhibitors also failed to show a significant clinical benefit.
- Namodenoson (CF102, Cl-IB-MECA; 8%); in 52% of cases, the disease is diagnosed at stage IV.

METHODS

Cells and Reagents
All experiments were performed with the pancreatic adenocarcinoma BxPC-3 cell line; namodenoson was prepared by WuXi. It was dissolved in DMSO to yield a stock solution of 10 mM.

RESULTS

Fig. 1. Namodenoson: Chemical structure.

Fig. 2. Inhibition of BxPC-3 cell growth by namodenoson (A) was mitigated by the A3AR antagonist MRS1523 (B). Error bars represent SE. *P<0.001, **P<0.01 (t-test vs control).

Fig. 3. Inhibition of BxPC-3 cell growth by namodenoson in nude mice. Error bars represent SE.

Fig. 4. The effect of namodenoson treatment (20 µM, 24 h) on protein expression in BxPC-3 cells as observed in Western blot analyses. (A) cell growth regulatory proteins downstream of A3AR (namodenoson); (B) Wnt/β-catenin signaling pathway proteins; (C) KRAS downstream proteins; (D) apoptotic proteins.

In Vitro Effects of Namodenoson on Pancreatic Carcinoma Growth
In vitro analysis using the Presto Blue assay demonstrated a significant dose-dependent inhibition of BxPC-3 cell growth upon treatment with namodenoson, which was mitigated by the A3AR antagonist MRS1523, showing that the effect of namodenoson was A3AR-mediated (Fig. 2).

In Vivo Effects of Namodenoson on Pancreatic Carcinoma Growth
- Analysis of the inhibitory effect of namodenoson (10 µg/kg) given twice daily for 35 days to nude mice inoculated with BX-PC3 cells demonstrated a significant inhibitory effect of namodenoson on tumor growth (inhibition of 67.7%±15.2% by day 57 vs controls, t-test) (Fig. 3).

In Vitro Effects of Namodenoson on Signal Transduction
- Western blot analysis using BxPC-3 cells demonstrated that namodenoson induced a decrease in A3AR expression level, and that the downstream regulatory proteins p-Akt, PI3K, and NF-κB were all down-regulated (Fig. 4A). Analysis of proteins within the Wnt signal transduction pathway revealed upregulation of GSK-3β, and a decrease in the expression levels of β-catenin, and cyclin D1 (Fig. 4B). A decrease in the expression levels of proteins downstream to the RAS signaling pathway (Raf, MEK 1/2, and ERK 1/2) was also observed (Fig. 4C). Additionally, the 2 apoptotic proteins Bax and Bax were up-regulated, suggesting that apoptosis of the BX-PC3 cells was induced (Fig. 4D).

CONCLUSIONS
- Namodenoson inhibits the growth of pancreatic carcinoma in vivo and in vitro in a mechanism that involves A3AR activation, de-regulation of the Wnt/β-catenin and the Ras signaling pathways, and activation of apoptosis.
- Our finding supports the continued development of namodenoson as a treatment for pancreatic cancer.

OBJECTIVES
The objectives of the current preclinical study were to examine the anti-growth effect of namodenoson on pancreatic carcinoma cells both in vitro and in vivo to investigate the molecular mechanisms involved.

METHODS

Cells and Reagents
- All experiments were performed with the pancreatic adenocarcinoma BxPC-3 cell line; namodenoson was prepared by WuXi. It was dissolved in DMSO to yield a stock solution of 10 mM.

In Vitro and In Vivo Studies
- Presto Blue assay was used to monitor cell growth after incubation of BxPC-3 cells (1x10⁴/mL) with 5, 10, or 20 nM namodenoson in 96-well plates for 24 h.
- Nude male Balb/C mice (Harlan Laboratories, Israel), aged 2 months (mean weight, 25 g), were administered a subcutaneous flank injection of BxPC-3 cells (2.5x10⁶). When the tumor reached a size of 150-200 mm³, the animals were randomly assigned to 2 groups each containing 10 animals (namodenoson, 100 µg/kg body weight given orally twice daily for 35 days, and control). Tumor size was measured twice weekly.
- Western blot analyses were performed (using the same conditions as the Presto Blue studies in 10-cm plates) to assess the expression levels of cell growth regulatory proteins using rabbit polyclonal antibodies against phosphorylated-PKβ/Akt (p-PKB/Akt), NF-κB, β-catenin, A3AR, PI3K, GSK-3β, cyclin D1, ERK 1/2, MEK 1/2, Raf, and Bax (Santa Cruz Biotechnology Inc., USA).
- In vivo experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at Can-Fite BioPharma (Israel).

REFERENCE

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