Efficacy of Diethyldihydroxyhomospermine Against Human Pancreatic Adenocarcinoma Using Orthotopic Implantation of Human Pancreatic L3.6pl Cells into The Pancreas of Nude Mice

Ajit K. Shah, Michael T. Cullen, Cheryl H. Baker

Sun BioPharma Inc., Ponte Vedra Beach, FL

Abstract #3128

Purpose: To determine the anti-neoplastic effects of (S,S)N1,N14-diethyl-3,12-dihydroxyhomospermine ($[HO]_2$DEHSPM, SUN-101) after subcutaneous (s.c.) administration after orthotopic transplantation of human pancreatic cancer cells (L3.6pl) in the pancreas of nude mice.

Methods: L3.6pl cells were injected into the pancreas of nude mice and seven to ten days later the treatment groups were:

Study 1: mice (n=10/group) with saline (control), SUN-101 (25 mg/kg), SUN-101 (50 mg/kg), and SUN-101 (100 mg/kg) QD for 4 to 6 weeks.

Study 2: mice (n=10/group) with saline (control), SUN-101 (25 mg/kg, QD), SUN-101 (25 mg/kg three times a week, QOD), SUN-101 (15 mg/kg, QOD), and SUN-101 (5 mg/kg, QOD) for 4 to 6 weeks

Study 3: mice (n=10/group) with saline (control), gemcitabine (100 mg/kg, intraperitoneal (i.p.), twice a week), SUN-101 (25 mg/kg, QOD), and gemcitabine plus SUN-101 for 4 to 6 weeks.

Results: In study 1, the optimal dose of SUN-101 was determined to be 25 mg/kg QD. This dose reduced up to 82.9% the weight of human pancreatic tumors in mice. Treatment with 50 and 100 mg/kg doses resulted in decreased body weight and proved to be toxic in mice. Histologic changes in the liver included hepatocyte reparative change and in the exocrine pancreas included a mild decrease of cytoplasmic granules in the epithelium of the pancreatic acini.

In study 2, the optimal dose of 25 mg/kg administrated QOD was less toxic than daily 25 mg/kg administrations. The pancreatic weight and volume were decreased (47.8% and 66.6%, respectively) with 25 mg/kg administrated QOD and dose related decreases were observed at the 15 mg/kg QOD (20.1% and 52.6%, respectively). No decrease in tumor weight or volume was noted with 5 mg/kg administrated QOD.

In study 3, the treatment of gemcitabine, SUN-101, and gemcitabine plus SUN-101 resulted in 18.7%, 35.6%, and 42.4% decreases in the body weight, respectively. Compared with tumor-bearing control, the treatment of gemcitabine, SUN-101, and gemcitabine plus SUN-101 resulted in 24.7%, 58.8%, and 67.2% decreases in the pancreas weight, respectively, and 37.8%, 58.4%, and 72.9% decreases in the tumor volumes, respectively.

The incidence of liver metastasis was also decreased with SUN-101, gemcitabine, and combination of gemcitabine plus SUN-101.

Conclusions: SUN-101 administered QD or QOD 25 mg/kg inhibited the growth of human pancreatic carcinoma in mice. SUN-101 demonstrated higher toxicity including disruption of the digestive process at daily 50 and 100 mg/kg doses. Co-administration of SUN-101 with gemcitabine appeared to have an additive or synergistic effect on reduction in the pancreatic tumor.

Introduction

The polyamine metabolic pathway has been widely investigated as a potential target for cancer treatment since the discovery that polyamine metabolism is frequently dysregulated in neoplastic disease[1-4]. A hydroxylated derivative of the polyamine analog, diethydihydroxylhomospermine ($[HO]_2$DEHSPM, SUN-101), was tested in dogs and the most notable effect was the profound and selective destruction of the exocrine pancreas.

Objectives

The anti-neoplastic effects of SUN-101 after subcutaneous (s.c.) administration were determined using human pancreatic cancer cells (L3.6pl) grown orthotopically in the pancreas of nude mice[5-9]. Three studies were conducted as follows:

- Study 1: to determine the efficacy of SUN-101 at 25, 50, and 100 mg/kg daily (QD) s.c. doses for 4 to 6 weeks in tumor-bearing and non-tumor-bearing nude mice.
- Study 2: to determine the efficacy of SUN-101 at 5, 10, and 25 mg/kg three times a week (QOD) s.c. doses for 4 to 6 weeks in tumor-bearing and non-tumor-bearing nude mice.
- Study 3: to determine the efficacy of SUN-101 at 25 mg/kg QOD s.c. dose for 4 to 6 weeks in combination with Gemzar (gemcitabine) at 100 mg/kg twice a week i.p. dose in tumor-bearing mice

Methods

- Mice were injected into the pancreas with 1 million L3.6pl pancreatic cancer cells and tumors were allowed to develop over 7-10 days.
- Non-tumor-bearing nude mice served as control and were not injected with L3.6pl cells. The control mice were treated in the same way as the tumor-bearing mice.

Table 1: Study 1 dosing

I. Non-tumor-bearing mice			II. Tumor-bearing mice		
Group	Dose	Number of animals	Group	Dose	Number of animals
Control	Saline (PBS)	10 mice	Control	Saline (PBS)	10 mice
SUN-101	25 mg/kg QD	5 mice	SUN-101	25 mg/kg QD	5 mice
SUN-101	50 mg/kg QD	9 mice	SUN-101	50 mg/kg QD	9 mice
SUN-101	100 mg/kg QD	9 mice	SUN-101	100 mg/kg QD	7 mice

Table 2: Study 2 dosing

I. Non-tumor-bearing mice			II. Tumor-bearing mice		
Group	Dose	Number of animals	Group	Dose	Number of animals
Control	Saline (PBS)	10 mice	Control	Saline (PBS)	8 mice
SUN-101	25 mg/kg QOD	10 mice	SUN-101	25 mg/kg QOD	8 mice
SUN-101	15 mg/kg QOD	10 mice	SUN-101	15 mg/kg QOD	10 mice
SUN-101	5 mg/kg QOD	4 mice	SUN-101	5 mg/kg QOD	10 mice
SUN-101	25 mg/kg QD	1 mouse	SUN-101	25 mg/kg QD	2 mice

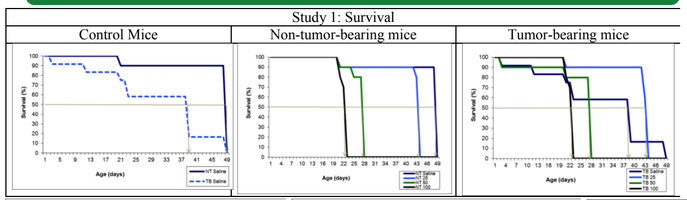
Table 3: Study 3 dosing

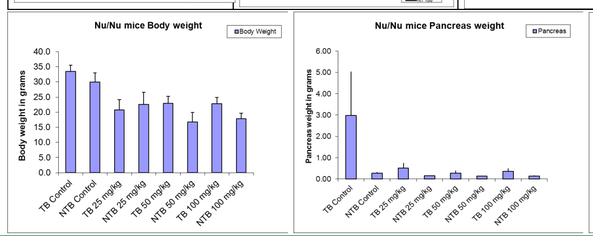
Tumor-bearing mice		
Group	Dose	Number of animals
Control	Saline (PBS)	7 mice
Gemzar[1]	100 mg/kg	6 mice
SUN-101[2]	25 mg/kg	9 mice
SUN-101[2]**+ Gemzar**[1]	25 mg/kg 100 mg/kg	10 mice

[1]: i.p. twice a week dosing
[2]: s.c. three times a week dosing

Results

Study 1: Survival





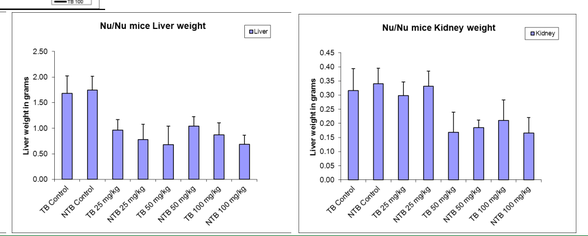
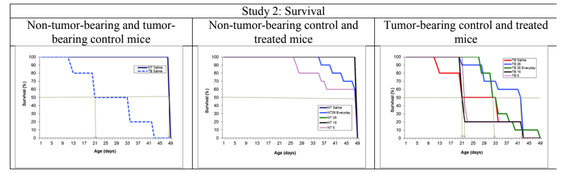


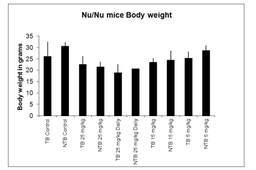
Table 4: Histologic results in tumor-bearing mice

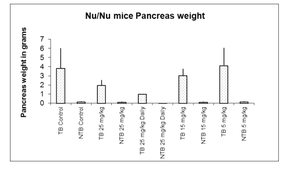
Tumor-bearing mice	Pancreatic tumor size mm^3 (mean)	Liver metastases	Reparative change in Liver	Decrease of exocrine pancreatic granules
No drug Mouse no. 7	Early 467 Mid 2352 Late 2097	4 out of 7	----	**----**
25 mg/kg Mouse no. 4	419	2 out of 4	none	**3 out of 4**
50 mg/kg Mouse no. 7	235	1 out of 7	4 out of 7	**2 out of 7**
100 mg/kg Mouse no. 7	200	1 out of 7	7 out of 7	**2 out of 7**

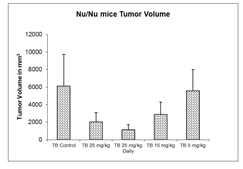
Results

Study 2: Survival

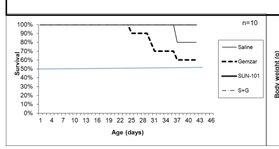


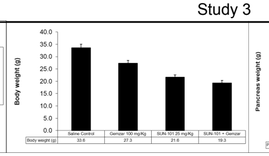


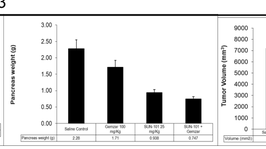


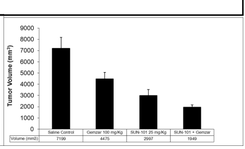


Study 3









Conclusions

- The administration of graduated doses of SUN-101 at 15 mg/kg, 25 mg/kg, 50 mg/kg, and 100 mg/kg resulted in inhibition of pancreatic tumor growth and decreased metastasis to other organs; the 5 mg/kg dose was found to be ineffective.
- All tumor-bearing mice that received SUN-101 at 15, 25, 50 and 100 mg/kg dosage demonstrated decreases in pancreas weights and volume of tumors compared with the matching controls without SUN-101.
- The optimal biological dose was determined to be 25 mg/kg daily (QD) or three times a week (QOD) based on inhibition of tumor growth, drug tolerance, survival, and histologic effects.
- Co-administration of SUN-101 at 25 mg/kg dose three times a week with gemcitabine at 100 mg/kg dose twice a week appeared to have an additive or synergistic effect on the reduction in pancreatic tumor.

References

1. Hector S, Tummala R, Kisiel ND, Diegelman P, Vujcic S, Clark K, Fakih M, Kramer DL, Porter CW, Pendyala L: Polyamine catabolism in colorectal cancer cells following treatment with oxaliplatin, 5-fluorouracil and N1, N11 diethylnorspermine. Cancer Chemother Pharmacol 2008;62:517–527.
2.Jänne J, Pösö H and Raina A: Polyamines in rapid growth and cancer. Biochim Biophys Acta 1978;473:241–293.
3.Porter CW, Bergeron RJ: Enzyme regulation as an approach to interference with polyamine biosynthesis - an alternative to enzyme inhibition. Adv Enz Reg 1988;27:57–79.
4.Casero RA Jr, Wang Y, Stewart TM, Devereux W, Hacker A, Wang Y, Smith R, Woster PM: The role of polyamine catabolism in anti-tumour drug response. Biochem Soc Trans 2003;31:361–365.
5.Bruns CJ, Harbison MT, Kuniyasu H, Eue I, Fidler IJ. In vivo selection and characterization of metastatic variants from human pancreatic adenocarcinoma by using orthotropic implantation in nude mice. Neoplasia, 1999, 1:50-62.
6.Baker CH, Solorzano CC, Fidler IJ. Angiogenesis and Cancer Metastasis: Antiangiogenic Therapy of Human Pancreatic Adenocarcinoma. Int J Clin Oncol. 2001;6:59-65.
7.Baker CH, Solorzano CC, Fidler IJ. Blockade of Vascular Endothelial Growth Factor Receptor and Epidermal Growth Factor Receptor Signaling for Therapy of Metastatic Human Pancreatic Cancer. Cancer Res. 2002;62:1996-2003
8.Baker CH, Trevino JG, Summy JM, Zhang F, Caron A, Nesbit M, Gallick GE, Fidler IJ. Inhibition of PDGFR Phosphorylation, Src and Akt Activities by GN963 Leads to Therapy of Human Pancreatic Cancer Growing Orthotopically in Nude Mice. Int J Clin Oncology. 2006;29:125-38.
9.Konduri SD, Ticku J, Robustuc GC, Sutphin RM, Colon J, Bhakat KK, Kalkunte SK, Baker CH. Blockade of MGMT Expression by O6 Benzyl Guanine Leads to Inhibition of Pancreatic Cancer Growth and Induction of Apoptosis. Clin. Cancer Res., 2009:15:6087-6095

This document contains proprietary confidential information of the Sun BioPharma Inc. and may not be disclosed verbally, abstracted, or published without written consent

Printed by Call4Posters