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PCK-3145

Prostate Cancer Therapy

PSP-94 (31-45)

L-Glutamyl-L-tryptophyl-L-glutaminyl-L-threonyl-L-aspartyl-L-asparaginyl-L-cysteinyl-L-glutamyl-L-threonyl-L-cysteinyl-L-threonyl-L-cysteinyl-L-tyrosyl-L-glutamyl-L-threonine

31-45-β-Inhibin (human)

$C_{73}H_{104}N_{18}O_{31}S_3$
Mol wt: 1825.9076

CAS: 414903-37-0

EN: 308234

Abstract

Prostate secretory protein (PSP-94) is one of the dominant proteins in human semen that is synthesized and secreted by the prostate gland. However, its synthesis is not exclusive to the prostate and it is also produced by other human tissues. Abundant amounts of PSP-94 are produced in normal prostatic epithelial tissue and benign prostatic hyperplasia tissue, while levels of expression are markedly reduced in patients with advanced prostate cancer. These findings suggested the potential of PSP-94 as a therapeutic agent for late-stage hormone-refractory prostate cancer (HRPC). Structure-function studies showed that the antiproliferative, proapoptotic and antitumor effects attributed to PSP-94 can be localized to the region corresponding to amino acids 31-45 (PCK-3145). Syngeneic and xenograft studies using various models of prostate cancer demonstrated the ability of both PSP-94 and PCK-3145 to reduce tumor growth *in vivo*. More importantly, PCK-3145 was able to reduce the development of skeletal metastasis associated with prostate cancer. Additionally, PCK-3145 reduced tumor parathyroid hormone-related peptide (PTHrP), which is the main pathogenic factor responsible for hypercalcemia of malignancy associated with several cancers, including prostate cancer. The antitumor effects of PCK-3145 were attributed to its ability to promote tumor cell apoptosis. Collectively, these studies led to the clinical development of PCK-3145. After successful completion of the first human clinical trials in the U.K., PCK-3145 is currently being studied in pilot phase I trials in patients with HRPC in the United States.

Synthesis

PCK-3145, corresponding to amino acids 31-45 of PSP-94 (Fig. 1), was prepared using solid-phase synthesis which involved the following steps: assembly and synthesis of the peptide on resin using Fmoc chemistry, side-chain deprotection and cleavage of the peptide from the resin using trifluoroacetic acid (TFA), and purification of the crude peptide using reverse-phase high-performance liquid chromatography (RP-HPLC).

Introduction

Human prostate cancer is a common hormone-dependent cancer associated with a high incidence of morbidity and mortality. While early detection, hormone therapy and newly approved chemotherapeutic agents have had beneficial effects, later stage hormone-refractory prostate cancer (HRPC) continues to be a challenging clinical problem (1-3). In the later stages, tumor cells are hormone (androgen)-nonresponsive and highly invasive. This change in tumor cell behavior is attributed to the silencing of tumor suppressor genes and activation of tumor-promoting genes, which include a variety of growth factors, cytokines and proteases (4-7). Identification and characterization of these proteins, which are differentially expressed in normal tissue, early- and late-stage cancer, have resulted in the identification of genes that can be targeted for diagnostic, prognostic and therapeutic development.

Using a number of cellular and molecular biology techniques, differential expression of PSP-94 has been demonstrated in different stages of human prostate cancer (8, 9). These studies showed that PSP-94 is highly

S.A. Rabbani[1]*, N. Shukeir[1], S. Garde[2], J.J. Wu[2], C. Panchal[2]. [1]Department of Medicine, Physiology and Oncology, McGill University Health Centre, Montreal, Quebec, Canada; [2]Procyon BioPharma, Inc., Montreal, Quebec, Canada. *Correspondence: e-mail: shafaat.rabbani@mcgill.ca.

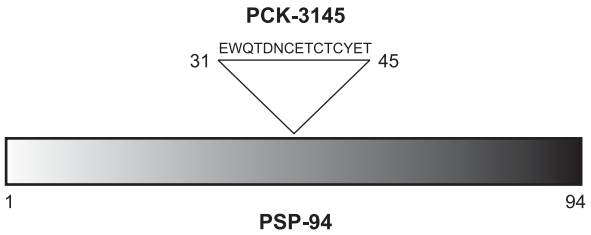

Fig. 1. Amino acid sequence of PCK-3145.

expressed in normal prostatic tissue, while levels of expression are reduced in patients with prostate cancer. Along with other genes which are well characterized in prostate cancer, such as prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP), PSP-94 can also be developed as a diagnostic/prognostic marker for prostate cancer (10, 11). However, unlike PSA and PAP, the regulation of which is androgen-dependent, the production of PSP-94 is androgen-independent, which allows its use as a marker in patients who have received androgen ablation therapy (12, 13).

While PSP-94 was initially characterized as a protein expressed in the prostate gland, like PSA, its expression is not restricted to the prostate gland (14). Indeed, several other tissues have been shown to express PSP-94, thereby broadening its scope as a diagnostic and therapeutic agent for other common malignancies, including gastric and breast cancer (14, 15). A knock-in model of PSP-94 showed early development of prostate cancer in mice at puberty. This PSP knock-in mouse adenocarcinoma prostate model (PSP-KIMAP) revealed tumor metastasis to lungs, liver and lymph nodes, further validating its role in prostate cancer progression (16).

Although a bona fide PSP-94 receptor has yet to be identified and characterized, a novel human protein in blood that can selectively bind PSP-94 (PSPBP) has been identified (17). This novel PSPBP is expressed in blood leukocytes, the prostate, testes, ovaries and intestine, as demonstrated by Northern blot analysis. Additional immunohistochemical analysis showed its expression in the pituitary and Leydig cells, suggesting a role in the pituitary-gonadal axis. Indeed, studies have shown the ability of PSP-94 to suppress the expression of follicle-stimulating hormone (FSH) (18, 19). Interestingly, higher expression of FSH receptors in patients with benign prostatic hyperplasia (BPH) and the demonstration of bona fide FSH receptors in the prostate gland led to the proposed autocrine/paracrine role of FSH in prostate cancer (20-22).

Pharmacological Actions

The pharmacological actions of PSP-94 have been evaluated in both syngeneic and xenograft models of prostate cancer. The use of syngeneic models provides an opportunity for the evaluation of the therapeutic effects of PSP-94 in a system where full interaction between tumor cells and the surrounding microenvironment is observed.

Evaluation of the therapeutic effects of PSP-94 was carried out using a syngeneic rat model of prostate cancer where rat prostate cancer MatLyLu cells are transfected with full-length cDNA encoding PTHrP (MatLyLu-PTHrP). PTHrP is known to be the major pathogenic factor responsible for the hypercalcemia seen in 15-20% of patients with malignancy, including patients with late-stage HRPC (23-27). *In vitro* analyses revealed that PSP-94 reduced the proliferative rate of these cells due to its ability to induce apoptosis. For *in vivo* studies, MatLyLu-PTHrP cells were injected subcutaneously (s.c) or via intracardiac (i.c.) injections into the left ventricle of male Copenhagen rats. Subcutaneous injections resulted in the development of primary tumors, whereas i.c. injections resulted in the development of experimental skeletal metastases in lumbar vertebrae, a complication often seen in patients with late-stage prostate cancer. Administration of PSP-94 resulted in a dose-dependent decrease in primary tumor growth and the highest dose tested led to a significant delay in the development of hindlimb paralysis, which is indicative of the development of experimental skeletal metastases. Furthermore, PSP-94 was able to reduce the levels of tumor and plasma PTHrP, as determined by radioimmunoassay of plasma from control and experimental animals and by immunohistochemical analysis of primary tumors. This decrease in tumor PTHrP production led to a dose-dependent decrease in plasma calcium levels in animals receiving PSP-94 (28).

In order to determine the minimum amino acid regions responsible for the antitumor effects of PSP-94, structure-function studies were carried out using peptides spanning different regions of PSP-94. Only the peptide spanning amino acids 31-45 (PCK-3145) showed antiproliferative effects *in vitro*. Similar to PSP-94, PCK-3145 was able to decrease tumor growth in a dose-dependent manner, as well as delay the development of skeletal metastases. The delay in skeletal metastases was due to the ability of PCK-3145 to decrease the tumor burden in the lumbar vertebrae (29). In addition, PCK-3145 decreased hypercalcemia of malignancy due to its ability to decrease tumor PTHrP and plasma PTHrP levels (Figs. 2-4).

Although the use of syngeneic models provides both a rapid and effective method for evaluating the therapeutic effects of PSP-94 and PCK-3145, the use of xenograft models is warranted, as these models represent a system that mimics the progression of the disease in humans. Studies have been carried out with human prostate cancer PC-3 cells *in vitro* and using xenograft models of prostate cancer *in vivo*. In these studies, PSP-94 was able to decrease PC-3 cell proliferation and tumor volume due its ability to induce tumor cell apoptosis (30). Further studies are needed to evaluate the antitumor effects of PCK-3145 in xenograft models of prostate cancer.

The results obtained in preclinical studies demonstrated the ability of PSP-94 and its synthetic analogue PCK-3145 to effectively reduce late-stage prostate can-

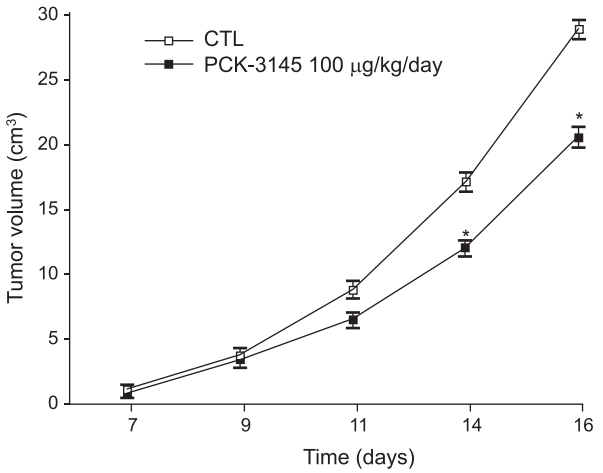

Fig. 2. Effect of PCK-3145 on MatLyLu-PTHrP tumor volume. Male Copenhagen rats were injected s.c. into the right flank with 1 x 10^6 MatLyLu-PTHrP cells. Starting from the day of tumor cell inoculation, animals were treated daily with PCK-3145 for 15 consecutive days. Tumor volume was measured at timed intervals and compared with tumor-bearing control (CTL) animals receiving vehicle alone. Results represent the mean ± SEM of 5 animals in each group in 3 different experiments. Significant differences from tumor-bearing CTL animals receiving vehicle alone are represented by asterisks ($p < 0.05$).

cer growth and metastasis, without manifesting any noticeable side effects. Additional studies to evaluate the efficacy of PCK-3145 in combination with other chemotherapeutic agents to block the progression of prostate cancer are in progress.

Pharmacokinetics

Pharmacokinetic (PK) studies with PCK-3145 were performed in mice, rats and cynomolgus monkeys. Mice receiving single doses of 10 or 100 μg/kg of PCK-3145 by bolus i.v. injection showed a profile of peptide serum concentrations *versus* time that was best described by a compartmental model. The clearance values were 2.8 and 3.4 l/h/kg at doses of 10 and 100 μg/kg, respectively, and the half-life values were 0.6 and 9.1 min, respectively. Copenhagen rats (18 per group) were also used to investigate the pharmacokinetics of PCK-3145 following a single i.v. bolus injection of 1000, 2000 or 4000 μg/kg. The pharmacokinetic profile of PCK-3145 was again best described using a compartmental model. Elimination was rapid, as indicated by elimination half-life values of 0.23-0.30 h. The pharmacokinetics of PCK-3145 appeared to be linear over the dose range 1000-4000 μg/kg. The pharmacokinetics of PCK-3145 were further investigated in cynomolgus monkeys (*Macaca fascicularis*) given single and repeated i.v. bolus injections at dose levels of 2.5, 10 and 25 mg/kg/day. The pharmacokinetic profile of PCK-3145 was described by a 2-compartment model. There was no accumulation following repeated administration for 28 consecutive days, which was attributed to the rapid clearance of PCK-3145. The elimination half-life values ranged from 0.21 to 0.33 h.

Clinical Studies

Based on the preclinical data from animal models suggesting that PCK-3145 reduces prostate tumor

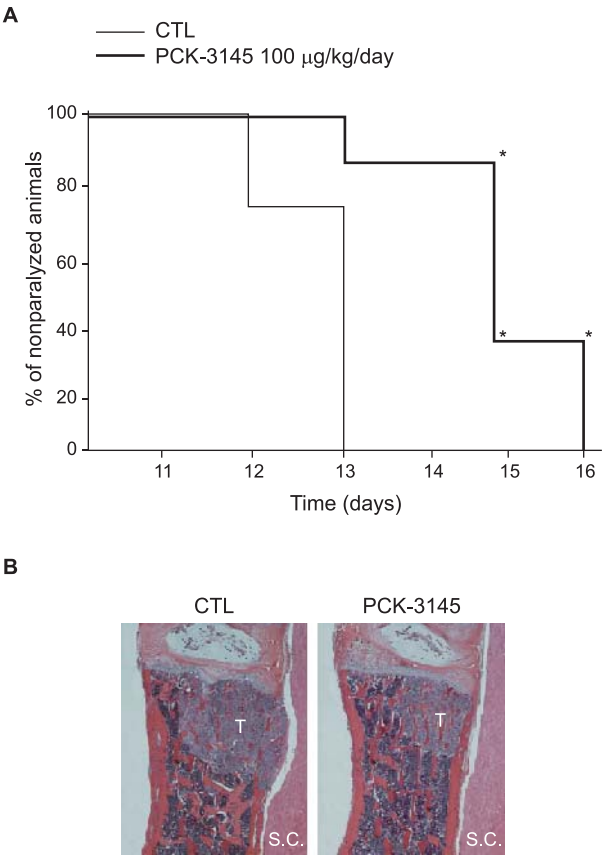

Fig. 3. Effect of PCK-3145 on experimental skeletal metastases. **Panel A:** Male Copenhagen rats were inoculated by intracardiac injection into the left ventricle with 10 x 10^3 MatLyLu-PTHrP cells. From the day of tumor cell inoculation, animals were infused daily with vehicle or PCK-3145 as described. The effect on the development of hindlimb paralysis was evaluated daily. The % of nonparalyzed animals at different time points receiving vehicle alone as control (CTL) or 100 μg/kg of PCK-3145 is shown. **Panel B:** Following the inoculation of MatLyLu-PTHrP cells via the intracardiac route into the left ventricle, animals were infused with vehicle alone or the highest dose of PCK-3145 (100 μg/kg/day) starting from the day of tumor cell inoculation for up to 10 days. All animals were sacrificed on day 10 and their vertebrae were removed, decalcified, paraffin-embedded and sectioned. Histological sections of vertebrae from animals receiving vehicle alone or PCK-3145 were stained with H & E. A representative photomicrograph of 3 such experiments is shown. Magnification 200X. Results represent the mean ± SEM of 5 animals in each group in 3 different experiments. Significant differences in the % of nonparalyzed animals from tumor-bearing CTL animals receiving vehicle alone are represented by asterisks ($p < 0.05$).

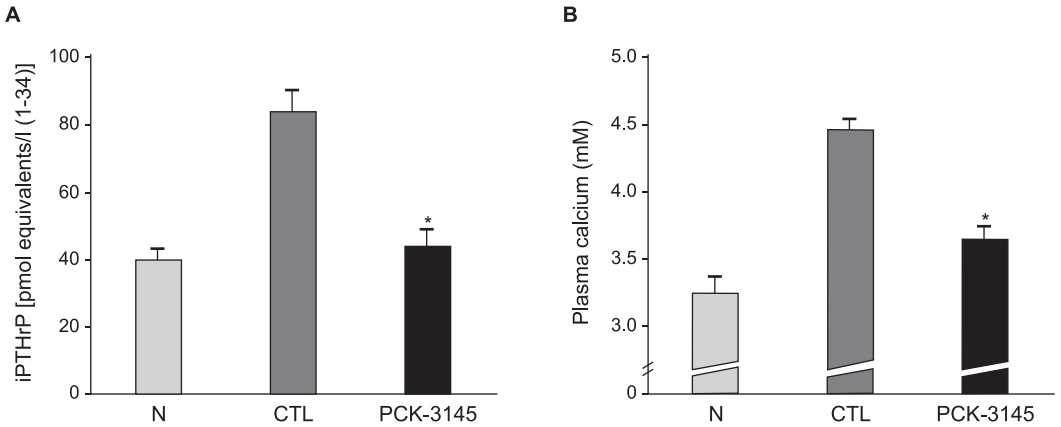

Fig. 4. Effect of PCK-3145 on plasma PTHrP and calcium in tumor-bearing animals. Male Copenhagen rats were inoculated s.c. with 1 x 10^6 MatLyLu-PTHrP cells. Starting at the time of tumor cell inoculation, animals were administered PCK-3145 (100 μg/kg/day) for 15 consecutive days. All animals were sacrificed at the end of the study (day 16) and plasma was collected from vehicle-treated control (CTL) animals and PCK-3145-treated animals and analyzed for immunoreactive plasma PTHrP (iPTHrP) (**panel A**) levels or plasma calcium (**panel B**). Plasma PTHrP and calcium levels in normal non-tumor-bearing animals are also shown (N). Results represent the mean ± SEM of 5 animals in each group in 3 different experiments. Significant differences from tumor-bearing CTL animals receiving vehicle alone are represented by asterisks ($p < 0.05$).

growth, a dose-escalating study was conducted in 16 patients with metastatic HRPC. Four cohorts of 4 patients received doses of 5, 20, 40 or 80 mg/m^2 i.v. One cycle consisted of administration of PCK-3145 3 times per week for 4 weeks followed by a 7-day rest period. On average, patients received 2 cycles, although 1 patient received 5 cycles without toxicity. The mean elimination half-life ranged from 0.354 to 1.45 h at doses of 20-80 mg/m^2. Clearance appeared to increase with increasing dose, but the mean AUC values did not increase proportionally with dose over the dose range studied. Patients remained under continued observation for PSA assessment and radiographic progression, with 3 patients having stable disease for 2 cycles. Overall, 7 patients had stable disease and 7 patients had progressive disease at the end of the study. One patient had an unconfirmed partial response. The biological activity of PCK-3145 was monitored by assessing the levels of MMP-9, MMP-2, TIMP-1 (tissue inhibitor of metalloproteinase-1), PSA, PAP, CEA (carcinoembryonic antigen), NSE (neuron-specific enolase) and hCG (human chorionic gonadotropin) in patient blood samples. A substantial reduction in MMP-9 levels was observed in 7 patients with elevated MMP-9 levels at baseline; 6 patients with low baseline MMP-9 levels (< 100 μg/l) had no change after the first cycle.

Future Directions

The mechanism of action of PCK-3145 and its effect as a single agent and in combination with other therapeutic drugs to block cancer growth and skeletal metastasis are currently being evaluated. For these studies, xenograft models of human prostate cancer using the human HRPC cell line PC-3 will be used. Following inoculation of tumor cells into the flank or tibia in male athymic nude mice, the effect of different doses of PCK-3145 on tumor growth and skeletal metastasis will be evaluated. Follow-up studies are also being planned to examine the efficacy of PCK-3145 alone or in combination with current therapeutic agents, such as docetaxel (Taxotere®). Additional efforts are under way to explore the molecular mechanism of action of PCK-3145, including its ability to downregulate MMP-9 gene expression, inhibit angiogenesis and modulate key intracellular signaling pathways implicated in tumor progression. Simultaneously, a pilot phase I trial is currently under way to explore the impact of PCK-3145 on MMP-9 levels. These studies may lead to the development of PCK-3145 as an effective therapeutic agent for patients with HRPC.

Acknowledgements

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Source

Procyon BioPharma, Inc. (CA).

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