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ORIGINAL ARTICLE

Orally administered FTS (salirasib) inhibits human pancreatic tumor growth in nude mice

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Abstract
Background *S-trans,trans*-farnesylthiosalicylic acid (salirasib, FTS) is a synthetic small molecule that acts as a potent Ras inhibitor. Salirasib inhibits specifically both oncogenically activated Ras and growth factor receptor-mediated Ras activation, resulting in the inhibition of Ras-dependent tumor growth. The objectives of this study were to develop a sensitive LC-MS/MS assay for determination of FTS in plasma, to assess the bioavailabilty of FTS after oral administration to mice, and then to examine the efficacy of orally administered FTS for inhibition of tumor growth in a nude mouse model.
Methods FTS was isolated from mouse plasma by liquid chromatography on a Columbus 5-μm particle size, 50 × 2 mm id column with a methanol/5 mM ammonium acetate (80/20) mobile phase (isocratic elution) at a flow rate of 0.3 ml/min. MS/MS was performed on a PE Sciex API 365 with Turbo Ion Spray as interface and negative ion ionization; parent ion (*m*/*z*): 357.2; daughter ion (*m*/*z*) 153.2; retention time 2.3 min. For plasma analysis, the amount of analyte in each sample was calculated by comparing response of the analyte in that sample to a nine-point standard curve linear over the range 3–1000 ng/ml. Pharmacokinetic studies were performed in mice following intraperitoneal dosing (20 mk/kg in PBS) or oral dosing (40 mg/kg in either 0.5% aqueous CMC or corn oil). Panc-1 tumor growth in nude mice was determined following daily oral dosing with FTS in 0.5% CMC (40, 60, or 80 mg/kg), or in combination with weekly gemcitabine (30 mg/kg).
Results Salirasib was readily detected in mouse plasma by LC-MS/MS at a detection limit of 3 ng/ml. For each route of administration, t_{max} was 1 h and $t_{1/2}$ ranged from 1.86 to 2.66 h. Compared to IP administration, the oral bioavailabilty of FTS was 69.5% for oral CMC and 55% for oral corn oil suspensions, while clearance and volume of distribution were higher in both oral preparations. The orally administered salirasib inhibited panc-1 tumor growth in a dose dependent manner (67% reduction in tumor weight at the highest dose, $P < 0.002$ vs. control, $n = 10$ mice per group) and at a 40 mg/kg daily dose was synergistic with gemcitabine (83% increase in survival rate, $n = 8$ mice per group).
Conclusions Salirasib exhibits good bioavailabilty after oral administration, as determined by a highly sensitive method for quantification in plasma. The orally available Ras inhibitor salirasib inhibited growth in nude mice, and may thus be considered for clinical trials.

Keywords FTS · Salirasib · Ras inhibitors · Oral salirasib · Pancreatic tumors

Introduction

S-trans,*trans*-farnesylthiosalicylic acid (FTS, salirasib) is a synthetic small molecule that acts as a potent Ras inhibitor [1–3]. It mimics the COOH-terminal farnesylcysteine carboxymethyl ester common to all Ras proteins which acts as part of a recognition unit for specific anchorage proteins that interact with Ras in the cell membrane and in endomembranes [2, 3]. The farnesyl moiety of Ras interacts

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with prenyl-binding pockets of Ras binding partners such as galectin-1 and galectin-3 [4–6], and these interactions support trafficking and targeting of Ras to distinctive cellular localities at which Ras activates signal transduction pathways. FTS interferes with the interactions of Ras with its binding partners, and this interference results in mislocalization of Ras as evident by dislodgment of Ras from the cell membrane, a major site from which Ras signals [6]. Once mislocalized by FTS, Ras protein can not activate the Raf/MEK/ERK and the PI3-K/Akt pathways [1, 7, 8] which play important roles in Ras transforming activity. Evidently, FTS exhibits high specificity towards the activated forms of all Ras proteins, including the oncogenically activated H-Ras[9], N-Ras [10] and K-Ras [11] isoforms, which are known to contribute to human malignancies [12–14]. This selectivity of FTS is attributable to the specific interactions of the active GTP-bound forms of Ras, not the inactive Ras-GDP forms, with their binding partners which are disrupted by FTS [2, 3, 7]. Experimental evidence point to the localization of Ras proteins to distinctive PM microdomains and in various intracellular compartments including the Golgi apparatus, the ER, and the mitochondria [15–17]. K-Ras is localized mainly to the plasma membrane while H-Ras and N-Ras exhibit significant plasma membrane and Golgi localization [15–17]. FTS, nonetheless, induces misslocaliztion of all oncogenic Ras isoforms independent of their cellular localities [2, 18, 19].

A host of studies have by now documented the ability of FTS to inhibit in vitro Ras-dependent growth and transformation in a variety of human tumor cell lines including pancreatic carcinomas [20], melanoma [10, 21], glioblastoma [22], ovarian carcinomas [23] colon carcinomas [24], prostate cancer cells [25] and malignant neurofibromatosis type 1 (NF1) cells [26]. Experiments in animal tumor models including melanoma SCID mouse models [27] and nude mouse models with panc-1 tumors [20]) and ST88 NF1 tumors [26] showed that intraperitoneally (IP) administrated FTS results in the inhibition of tumor growth. FTS is a non-toxic compound and exhibited no adverse side effects in animals [20, 27], most likely because it does not affect the inactive Ras proteins of normal cells and does not inhibit the growth of untransformed cells grown under normal conditions [9, 11]. In addition, under normal growth conditions FTS does not exhibit cytotoxic effects [9, 11, 26, 28].

All of these experiments support the notion that FTS might qualify as an anti-tumor drug. A major issue then is whether or not FTS is suitable for oral delivery. In the present study we describe the oral bioavailability of FTS, the pharmacokinetics of the orally administered FTS, and its ability to inhibit panc-1 tumor growth in a nude mouse model.

Materials and methods

Dose preparation and animals

Carboxymethylcellulose was obtained from Sigma (C4888). FTS (salirasib) was a gift from Concordia Pharmaceuticals (Sunrise FL). A solution of 0.5% carboxymethyl-cellulose (CMC) solution was prepared as follows. About 60 ml of double distilled water were added to 0.5 g CMC. After mixing for 4 h with a magnetic stirrer the solution was transferred to a volumetric flask and the volume was adjusted to 100 ml. The solution was kept at 4 degrees. A suspension of 5 mg/ml FTS in 0.5% CMC was prepared as follows: 150 mg FTS were added to a mortar and grind with a pestle. Few drops of the CMC were added and worked with the pestle. Gradually more CMC solution was added until the mixture became pourable. The suspension was poured into a volumetric flask. The mortar and pestle were rinsed few times with CMC solution that was collected in the volumetric flask. The volume was adjusted to 30 ml. The suspension was then stirred with a stirring bar for 1 h and aliquots of 3 ml were kept in vials at 4°C. FTS was stable in aqueous CMC for at least a month. Before drug administration, the suspension was warmed to room temperature and stirred continuously. FTS was also prepared for oral administration in corn oil (Sigma) by direct dispersion of the powder in the oil. For IP administration FTS was prepared in 10% ethanol and 90% PBS as described earlier [20, 26]. It was shown that FTS in this formula inhibits panc-1 tumor growth in mice at doses of 10–40 mg/kg [28]. For the pharmacokinetic experiments the concentration of FTS in the vehicle was such that a dosing volume of 10 ml/kg (oral) or 5 ml/kg (IP) body weight delivered the intended dose level. For experiments testing the impact of FTS on tumor growth, doses of 40, 60 or 80 mg/kg FTS in the CMC vehicle were used alone or in combination with gemcitabine. Gemcitabine was prepared in PBS as detailed earlier [29]. Control animals received the CMC vehicle only. CD1 male mice were used for the pharmacokinetic experiments and CD1-Nu nude mice were used for tumor growth experiments. The animals used in this study were obtained from Charles River Laboratories.

Pharmacokinetic sampling and analysis

Assay development and plasma concentration determinations were conducted by Ricerca Biosciences, LLC, Concord, OH. FTS oral bioavailabilty was calculated by comparing AUC after oral dosing with that after IP dosing. Eighteen animals were used to collect samples for each test article, three animals at each time point (1, 2, 4, 8, 12, and 24 h). Approximately 0.5 ml of blood was collected in lithium heparin containing tubes via the retro-orbital plexus

while under CO_2/O_2 anesthesia at each of the specified collection times. Following blood collection, the samples were centrifuged under refrigeration (2–8°C for 10 min at 1,500*g*) and the plasma was harvested into a single tube for each animal. The plasma was frozen at −70°C and then transferred for analysis. Peak plasma concentration, time peak achieved and half-life (C_{max}, t_{max} and $t_{1/2}$) were calculated. Area under the plasma concentration-time curves (AUC), volume of distribution and clearance were also determined. These analyses were conducted using WinNonlin (Phasight Corp).

Preparation of calibration standards and extraction of plasma for analysis

A stock solution of FTS was prepared in water at 124 µg/ml, and diluted with water to prepare a 10 µg/ml solution of FTS. Aliquots of these solutions were then used to prepare spiked calibration curve samples of FTS in control mouse plasma at 1000, 900, 500, 300, 30, 10, 4, 3 and 1 ng/ml.

Extraction procedure was as follows. Aliquots (100 µL) of plasma (study samples, spiked calibration curve samples) were mixed with 10 µl of water (samples) or fortified with 10 µl of appropriate standard solution (calibration standards), 50 µl of pH 11 buffer solution, vortex-mixed with 1 ml of acetonitrile to precipitate protein, and centrifuged at 14,000 rpm for 10 min. The supernatant was decanted from each sample into a clean centrifugation tube and evaporated to dryness with nitrogen at approximately 37°C. The residue was resuspended in 100 µl of 70:30 water:acetonitrile, vortexed for 1 min, centrifuged at 14,000 rpm for 10 min, and the supernatant was transferred to autosampler vials.

Liquid chromatography and MS/MS conditions

Liquid chromatography (LC) was performed on a Columbus 5-µm particle size, 50 × 2 mm id column. Mobile phase was methanol/5 mM ammonium acetate (80/20) (isocratic elution) at a flow rate of 0.3 ml/min, and the injection volume was 20 µl. MS/MS was performed on PE Sciex API 365 with Turbo Ion Spray as interface and negative ion ionization. Parent ion (*m*/*z*): 357.2; daughter ion (*m*/*z*) 153.2; retention time 2.3 min. For plasma analysis, the amount of analyte in each sample was calculated by comparing the response of the analyte in the sample to that of a nine-point standard curve (3–1000 ng/ml). Any sample that exceeded the upper range of the calibration curve was appropriately diluted with acetonitrile:water and reanalyzed.

Nude mouse panc-1 tumor model

Panc-1 cells were grown in DMEM/10% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 95% air/5% CO_2 as described in previous studies (19, 20, 26). The cells were detached by trypsin/EDTA, washed with PBS and resuspended as detailed previously. Nude CD_1-Nu mice (25–30 g) were housed in barrier facilities on a 12-h light/dark cycle. Food and water were supplied ad libitum. On day zero, 5×10^6 cells in 0.2 ml of PBS were implanted subcutaneously just above the right femoral joint. When palpable tumors were observed (7–12 days after cell implantation) the animals were divided randomly into the various experimental and control groups, and drug treatment was begun as indicated. Tumor volumes or weights were determined as described previously [20, 26].

Ras pull-down assays and immunoblotting procedures

Tumors were weighed and then homogenized (10% w/v) in lysis buffer as detailed previously [5]. Total amounts of Ras and of phospho-ERK were determined in samples (60 µg protein) of each lysate by SDS-PAGE followed by immunoblotting with pan-Ras Ab [4, 5] The apparent amounts of Ras-GTP in 800 µg protein of total tumor lysates were determined by the glutathione-S-transferase (GST)-RBD (Ras-binding domain of Raf) pull-down assay, as described elsewhere [5, 30, 31]. The pulled-down Ras-GTP was subjected to SDS-PAGE followed by immunoblotting with pan-Ras Ab. Protein bands were visualized by enhanced chemiluminescence and quantified by densitometry using ImageJ computer software (National Institutes of Health, Bethesda, MD).

Results

Pharmacokinetics of orally and intraperitoneally administrated FTS (salirasib)

CD-1 mice were used to assess the pharmacokinetics of FTS after administration by oral gavage or IP injection. The first day of dosing for each animal was designated day zero. FTS was administrated to 18 mice per group. Following dosing, blood samples were collected from the orbital plexus at 1, 2, 4, 8, 12 and 24 h; samples were then processed and analyzed by LC-MS/MS as described in Methods. A calibration curve for FTS was prepared ranging from 1 to 1,000 ng/ml and was linear over the range of 3–1000 ng/ml (Fig. 1a). FTS was readily detected in mouse plasma by LC-MS/MS at a detection limit of 3 ng/ml. Several of the plasma samples contained concentrations of the compound well above the top calibration curve. These samples were reanalyzed following appropriate dilution. Typical chromatograms for FTS plasma analysis are shown in Fig. 1b for oral dosing in CMC or in corn oil and for IP dosing. Compared with the expected retention time of the

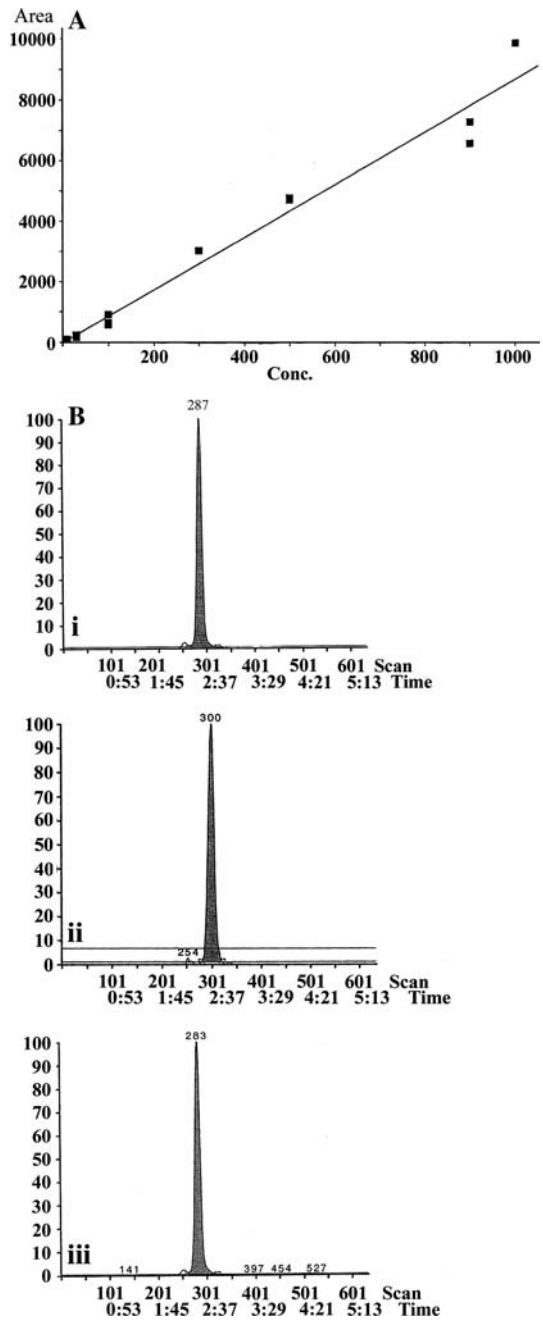

Fig. 1 Calibration curve and typical chromatograms for FTS plasma analysis. **a** Calibration curve. Serum was spiked with FTS to yield the indicated FTS concentrations. Samples were then processed and analyzed by LC-MS/MS as detailed in Methods. The areas under the peak recorded in each sample are plotted against the concentration of FTS (ng/ ml). Curve parameters: intercept = 157.0, slope = 87.1, correlation coefficient = 0.99. **b** Typical chromatograms for FTS plasma analysis of a 4 h sample of the oral CMC dosing (i), of an 8 h sample of the oral corn oil dosing (ii) and of a 2 h sample of the IP dosing (iii). Plasma samples were processed and analyzed by LC-MS/MS as detailed in "Materials and Methods"

parent ion (FTS, 2.23 min), the respective retention times of the tested sample ions were 2.29, 2.36 and 2.27 min, corroborating the assay method. Plasma concentration for FTS at the various time points of the CMC-40 mg/kg FTS oral dose, of the corn oil-40 mg/kg FTS oral dose and of the IP 20 mg/kg FTS dose are depicted respectively in Figs. 2, 3 and 4. These curves were then used to calculate the pharmacokinetic variables by a non-compartmental model as detailed in Methods. The results thus obtained namely, the terminal-phase half time (elimination half time, $t_{1/2}$), the time at which the maximum plasma concentration (C_{max}) was reached (t_{max}), the C_{max}, the area under the plasma concentration-time curve (AUC), the clearance and the volume of distribution are summarized in Table 1. Under all conditions t_{max} was 1 h where the $t_{1/2}$ ranged from 2.66 to 1.86 h and AUC was highest in the oral CMC formula. The highest C_{max} was recorded for the oral CMC preparation, while clearance and volume of distribution were high for both oral preparations as compared with IP administration. The bioavailability of FTS in mice, relative to the IP dose, was approximately 69.5% for the oral CMC and 55% for the oral corn oil doses (Table 1).

Taken together these experiments established a convenient method for the determination of plasma FTS and demonstrated that orally administrated FTS (salirasib) is

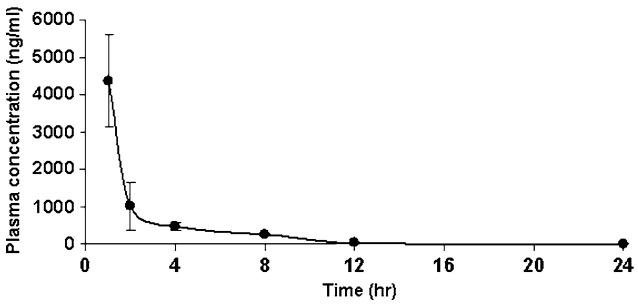

Fig. 2 Plasma concentration for FTS in oral dose 0.5% CMC. CD-1 mice received orally 40 mg/kg FTS in 0.5% CMC and the plasma concentration of FTS was determined at the indicated times by LC-MS-MS as described in Methods using the calibration curve shown in Fig. 1a. Mean values (±SD) determined at each time point ($n = 3$ mice per point) are shown

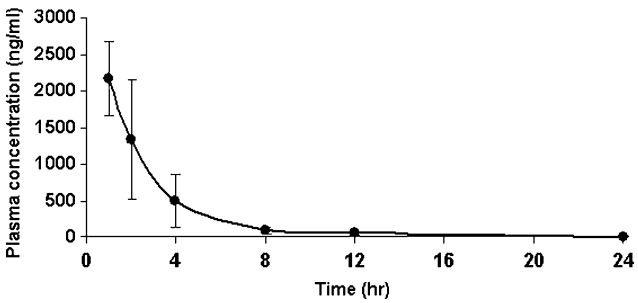

Fig. 3 Plasma concentration for FTS in oral dose in corn oil. CD-1 mice received orally 40 mg/kg FTS in corn oil and the plasma concentration of FTS was determined at the indicated times by LC-MS-MS as described in methods using the calibration curve shown in Fig. 1a. Mean values (±SD) determined at each time point ($n = 3$ mice per point) are shown

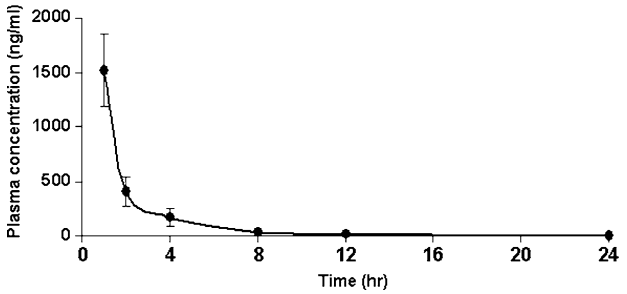

Fig. 4 Plasma concentration for FTS IP dose. CD-1 mice received 20 mg/kg FTS (IP) and the plasma concentration of FTS was determined at the indicated times by LC-MS-MS as described in methods using the calibration curve shown in Fig. 1. Mean values (±SD) determined at each time point (n = 3 mice per point) are shown

Table 1 Pharmacokinetic parameters for FTS in mice

Route of Administration	IP	Oral	Oral
Dose (mg/kg)	20	40	40
Vehicle	PBS-ethanol (9:1)	0.5 % CMC	Corn oil
Half-life (h)	1.86	2.66	2.36
T_{max} (h)	1	1	1
C_{max} (ng/mL)	1521.3	3381.1	2159.4
$AUC_{0\text{-inf}}$ (h ng/mL)	5672.2	7881.5	6207.2
Clearance (mL/h/kg)	3525.9	5075.2	6444.1
Volume of Distribution (mL/kg)	9477.2	19493.1	21959.7
Bioavailability (%)	100.0	69.5	54.7

Pharmacokinetic parameters in mice for FTS dosed IP or orally. Values were calculated using the mean time/concentration data at six time points (1 to 24 h). The oral bioavailablity of FTS in mice relative to IP dosing was 69.5% when administered in 0.5% CMC and 54.7% when administered in corn oil

well absorbed. The somewhat higher bioavailability of FTS in CMC as compared with corn oil led us to choose the CMC vehicle for the examination of the effect of oral FTS on tumor growth.

Oral salirasib (FTS) inhibits panc-1 tumor growth in a dose-dependent manner

We next examined the effect of oral salirasib (FTS) on panc-1 tumor growth using a nude mouse model. Panc-1 cells were implanted subcutaneously above the right femoral joint as described in Methods. Measurable tumors were observed 7 days after cell implantation (mean volumes 0.22–0.27 cm^3), and mice were then divided randomly into a control (10 mice) and FTS-treated (10 mice) groups. The control group mice received daily oral 0.5%CMC and the FTS treated mice received daily oral 40 mg/kg FTS in 0.5%CMC. Following a 10 day treatment period, the tumors were removed, weighed and homogenized for immunoblot assays. As shown in Fig. 5a, mean (±SD) tumor weight in the controls was 1.17 ± 0.46 g (n = 10) and in the 40 mg/kg FTS treated mice 0.56 ± 0.28 g (n = 10). Thus a statistically significant inhibition of tumor growth (52%, P = 0.0024) was recorded, indicating that the orally administrated FTS was effective.

The levels of total Ras, active Ras-GTP and phospho-ERK were then determined in homogenates of three tumors of the control and of the FTS treated mice as detailed in methods. The results (Fig. 5b) show that tumors of the FTS- treated mice exhibited lower levels of Ras, Ras-GTP and phospho-ERK as compared with controls. Data quantified by densitometry indicated that the FTS treatment caused a significant reduction in levels of total Ras (50%), of Ras-GTP (64%) and of phospho-ERK (84%). The results thus indicate that the oral 40 mg/kg dose of FTS was efficient in reducing the active Ras and its singling to the Raf/MEK/ERK cascade in the panc-1 tumors, consistent with the known mechanism of action of FTS as a Ras inhibitor [1, 7].

The next experiment was aimed at examining the dose-dependency of panc-1 tumor growth inhibition by oral salirasib (FTS). Panc-1 cell implantation was as detailed in Methods. Eight days after cell implantation tumors were observed (mean tumor volume 0.28–0.5 cm^3), the mice were divided randomly into 4 groups (10 mice per group), and daily treatments were begun. The groups were: 0.5%CMC controls, and 40 mg/kg, 60 mg/kg and 80 mg/kg oral FTS. Tumors were removed 21 days later and weighed. As shown in Fig. 5c, oral FTS treatment caused a dose-dependent inhibition of panc-1 tumor growth of 45, 59, and 67% tumor weight reduction, respectively at the 40, 60 and 80 mg/kg FTS doses.

The combined impact of gemcitabine and oral salirasib

The next experiment was aimed at examining the combined effect of gemcitabine and oral salirasib (FTS) on panc-1 tumor growth. Panc-1 cell implantation was as detailed in Methods. On day 7 after cell implantation tumors were observed (mean tumor volume 0.19–0.28 cm^3), and the mice were randomly divided into four groups (eight mice per group). Mice in group one received 0.5%CMC orally (control), in group two 40 mg/kg oral FTS, in group three 30 mg/kg gemcitabine (IP), and in group four 30 mg/kg gemcitabine and 40 mg/kg oral FTS. Treatment schedule was daily FTS and weekly gemcitabine for 3 weeks. Tumors were then removed and weighed. As shown in Fig. 6, the mean (±SD) tumor weight in controls was 2.65 ± 1.27 g and in the 40 mg/kg FTS treated animals 1.4 ± 0.58 g. Thus consistent with the results shown in Fig. 5a, oral 40 mg/kg FTS alone caused a statistically significant reduction in tumor weight (47%, P = 0.0044). Tumor weight in the gemcitabine treated mice was

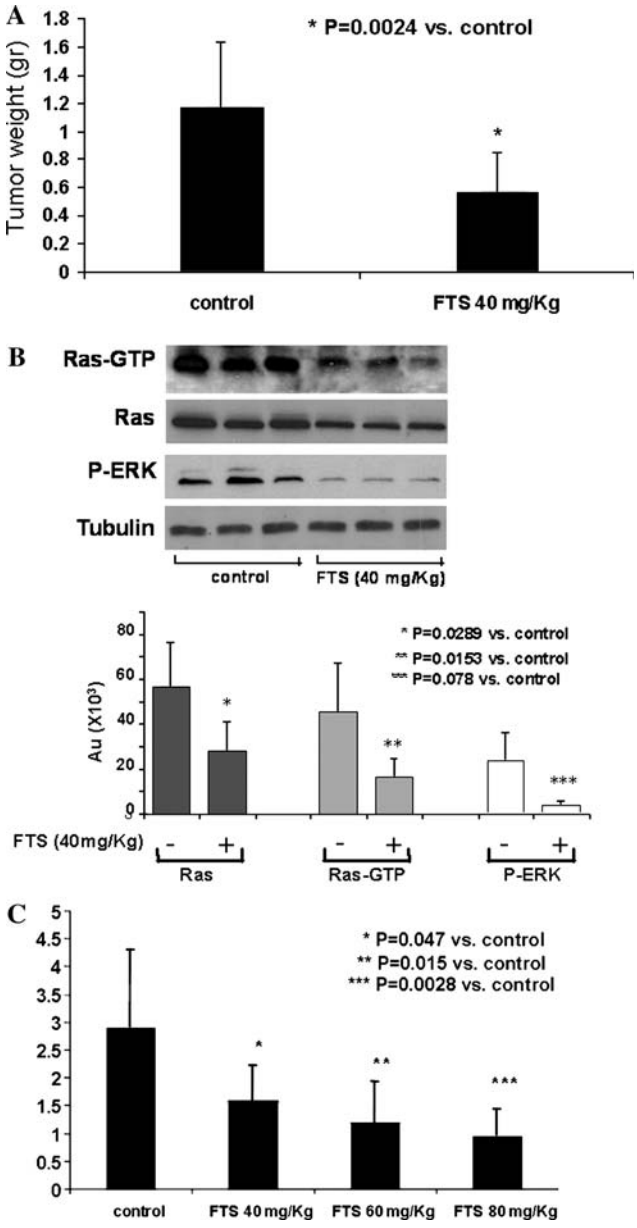

Fig. 5 Inhibition of panc-1 tumor growth in nude mouse by oral FTS. Panc-1 cells were injected subcutaneously into the flank areas of nude mice and when palpable tumors were observed the mice received daily an oral dose of 40, or 60, or 80 mg/kg salirasib in 0.5% CMC or 0.5% CMC (control) as detailed in the text. Tumors were removed after a period of 10 day (**a**, **b**) or 21 day (**c**) treatment, weighed and homogenized for the biochemical assays. **a** Mean tumor weights (±SD) recorded in the control ($n = 10$) and in the drug-treated mice ($n = 10$). *$P = 0.0024$. **b** Immunoblots of total Ras, Ras-GTP, and phospho-ERK. Samples of three control tumors and of three tumors of FTS-treated mice were subjected to the determination of total Ras, Ras-GTP and phospho-ERK as detailed in"Materials and Methods" . *Upper panel* immunoblots. *Lower panel* apparent levels of Ras, Ras-GTP and phospho-ERK determined by densitometry (AU) of the immonblots (mean ± SD values). *$P = 0.0289$, **$P = 0.0153$ ***$P = 0.078$. **c** Dose-dependent inhibition of panc-1 tumor growth in nude mice by oral FTS treatment. Mean (±SD) values of tumor weights recorded in the control, 40 mg/kg, 60 mg/kg and 80 mg/kg FTS treated mice are shown (10 mice per group). *$P = 0.047$, **$P = 0.015$, ***$P = 0.0028$ vs. control

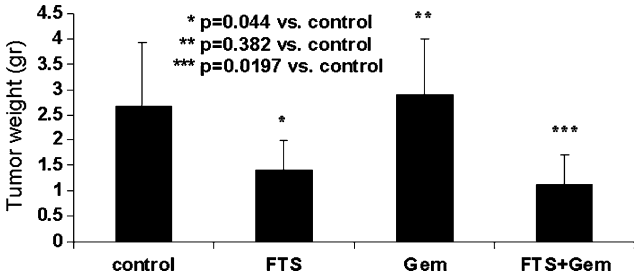

Fig. 6 The combined impact of gemcitabine and oral FTS on panc-1 tumor growth in nude mice. Panc-1 cells were injected subcutaneously into the flank areas of nude mice and when palpable tumors were observed the mice received daily an oral dose of 40 FTS in 0.5% CMC or 0.5% CMC (control), or weekly 30 mg/kg gemcitabine (IP) or a combination of daily FTS plus weekly gemcitabine. Tumors were removed after a period of 21 days and weighed. Mean (±SD) values of tumor weights recorded in the control and in the drug-treated mice as indicated (eight mice per group) are shown. *$P = 0.044$, **$P = 0.38$ (not significant) and ***$P = 0.019$ vs. control

2.9 ± 1.1 g, not significantly different than that recorded in the controls ($P = 0.382$), and in the combined treatment group 1.13 ± 0.58 g (Fig. 6). Thus the combination of drugs resulted in a significant ($P = 0.019$) 58% inhibition of panc-1 tumor growth. The combined gemcitabine and oral FTS treatment appeared to provide a stronger inhibitory effect than that provided by gemcitabine or oral FTS alone.

Oral salirasib (FTS) alone and in combination with gemcitabine increased the survival rate of panc-1 xenografted nude mice

The next set of experiments was conducted to determine the effect of oral FTS alone and in combination with gemcitabine on the survival rates of panc-1 xenografted nude mice. Four groups of mice receiving the drug combinations as described above were used in two separate experiments with distinct dosing schedule protocols. In the first experiment, treatment was daily oral FTS and weekly gemcitabine (IP) for 3 weeks, followed by one week interruption of drug treatment, followed by daily FTS and weekly gemcitabine for 3 weeks. The second experiment was with a similar schedule except that the oral FTS was not interrupted. In the first experiment the mean tumor volume of 32 mice on day 7 after cell implantation was 0.24–0.29 cm^3. The mice were randomly divided into four groups receiving the various treatments, and survival rates were recorded. Data presented as a Kaplan–Meier survival curve are shown in Fig. 7a. The half times of survival observed were 32.5 days in the control mice, 41 in the FTS-treated mice, 41 in the gemcitabine- treated mice and 59 in the FTS- plus gemcitabine-treated mice. Accordingly, the oral 40 mg/kg FTS or the gemcitabine treatments effected 27% increases in the

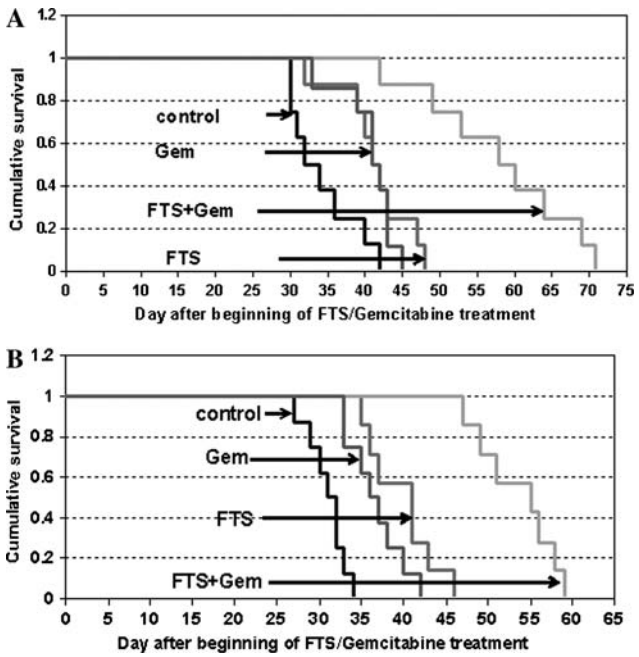

Fig. 7 Oral FTS alone and in combination with gemcitabin increased the survival rate of panc-1 xenografted nude mice. Panc-1 cells were injected subcutaneously into the flank areas of nude mice and when palpable tumors were observed the mice received daily an oral dose of 40 mg/kg FTS in 0.5% CMC or 0.5% CMC (control), or weekly 30 mg/kg gemcitabine or a combination of daily FTS plus weekly gemcitabine. A The treatment schedule was daily oral FTS and weekly gemcitabine (IP) for 3 weeks, followed by 1 week interruption of drug treatment, followed by daily FTS and weekly gemcitabine for 3 weeks. Data are presented as a Kaplan-Meier survival curve. **b** The treatment schedule was as in **a** except that the daily oral FTS was not interrupted. Data are presented as a Kaplan-Meier survival curve

survival rate of panc-1 tumor bearing mice, while the combined treatment resulted in an 83% increase. In the second experiment the mean tumor volume of 30 mice on day 7 after cell implantation was 0.19–0.32 cm^3, and the mice were randomly divided into four groups which received the treatments as described above. The results presented as a Kaplan–Meier survival curve are shown in Fig. 7b. The half times of survival observed were 31 days in control mice, 38.6 in FTS-treated mice, 36 in gemcitabine-treated mice and 52.4 in FTS-plus gemcitabine-treated mice. Accordingly, the oral 40 mg/kg FTS and the gemcitabine treatments effected respectively 24 and 16% increases in the survival rate of panc-1 tumor bearing mice, while the combined treatment resulted in a 69% survival rate increase. Together these experiments showed that oral FTS increased the survival rate by 24–27%, and that this treatment in combination with gemcitabine had a synergistic effect leading to a 69–83% increase in survival rate. The uninterrupted FTS treatment did not prolong the survival more than the interrupted FTS treatment.

Discussion

In the present study we established a method for the determination of plasma levels of salirasib (FTS), showed that FTS is orally available, and demonstrated that oral FTS effectively inhibits panc-1 tumor growth in a nude mouse model. We compared the pharmacokinetics of IP and of orally administered FTS, and showed that oral bioavailability of FTS is relatively high. Thus the oral bioavailability of FTS in mice, relative to IP dosing, was approximately 69.5% when administered orally in CMC and 55% when administered in corn oil (Table 1). Oral FTS rapidly reached peak plasma levels within 1 h (t_{max}), the $t_{1/2}$ of FTS ranged from 2.66 to 1.86 h, and AUC was highest for the oral CMC suspension. The highest C_{max}, was recorded in the oral CMC preparation, while clearance and volume of distribution were high in both of the oral preparations as compared with IP administration. Thus our results show that salirasib (FTS) can be used as an oral drug. Importantly, the panc-1 nude mouse experiments with oral salirasib indicated that this treatment was effective. Clearly oral salirasib effected a strong reduction in the level of active Ras and its downstream target phospho-ERK in the tumors and inhibited tumor growth. The inhibition of tumor growth was dose dependent and was comparable with that observed previously with IP administration of FTS [20, 26]. Moreover, in two separate experimental paradigms we found that the combination of salirasib and gemcitabine resulted in a synergistic effect leading to a significant increase in the survival rates of the tumor bearing mice. These results are important particularly in light of the lack of clinically meaningful survival benefit when gemcitabine was combined the farnesyl transferase inhibitor (FTI) tipifarnib [32]. A possible negativity of these trials is that FTIs lead to a G1-arrest and therefore might cause inactivation of gemcitabine.

Taken together, our results point to the potential of salirasib (FTS), the first orally available true Ras inhibitor, as a targeted anti-tumor drug. In line with the pharmacokinetic profile it is proposed that oral salirasib be given twice a day (BID). Clinical trials are now being conducted with capsules and tablets of salirasib with no reportable adverse side effects (http://www.concordiapharma.com/index.htm).

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