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Pilot Study of the Antitumor Efficacy and Tolerability of Orally Administered Rrx-001 in Normal and Tumor-Bearing Mice

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Abstract

Purpose RRx-001 is a novel anticancer drug that will be entering Phase III clinical trials as a pre-sensitizer or resensitizer to chemotherapy and radiotherapy. To date, it has been very well tolerated, with the main adverse event being infusion-related reactions, consisting primarily of discomfort, itching and tingling at the site of infusion. The purpose of this preclinical pilot study was to study the antitumor efficacy and tolerability of oral administration of RRx-001 in a preclinical mouse model.

Methods The maximum tolerated dose (MTD) of oral administration of RRx-001 was studied in normal C3H mice. For antitumor efficacy study, mice bearing SCC VII tumors were administered by oral gavage with RRx-001 either alone or in combination with external beam radiation therapy. Tumor growth delay time and body weight were used as the endpoints for antitumor efficacy and systemic toxicity.

Results The estimated MTD for oral RRx-001 in 10% dimethyl sulfoxide (DMSO) daily for 5 days was 10-20 mg/kg. In tumor-bearing mice, oral dosing of RRx-001 at a total equivalent dose of 60 mg/kg, given either daily, or every other day, or as a single dose, significantly inhibited tumor growth ($p < 0.01$ vs. vehicle control). There were no statistically significant differences in tumor growth delay time among three treatment regimens ($P > 0.05$). However, a single dose of 60 mg/kg caused a 28% modality (2 death among 7 treated mice). When combined with local tumor radiation therapy, oral RRx-001 significantly increased the antitumor efficacy of radiation therapy ($p = 0.02$, combination vs. radiation alone). There were no obvious systemic or additional toxicities for combination of oral RRx-001 (10-20 mg/kg) and radiation therapy.

Conclusion Oral administration of RRx-001 was safe and effective in the SCC VII tumor model in mice, and merits further study.

Keywords: RRx-001; anticancer drug; chemotherapy; efficacy; mouse tumor model

Introduction

RRx-001 (1-bromoacetyl-3, 3-dinitroazetidine, also known as ABDNAZ) is a first-in-class minimally toxic anticancer agent with multiple mechanisms of action and synergistic activity in combination with radiation and chemotherapy [1-3]. It will be advanced to Phase III clinical trials shortly for patients with advanced heavily pretreated refractory cancers including small cell lung cancer (SCLC), high-grade neuroendocrine carcinomas (HGNEC) and platinum-resistant ovarian cancer. In addition to anticancer activity, RRx-001 has also shown promise as a chemoprotector and radioprotector. When intravenously administered, RRx-001 rapidly binds to the reduced form of glutathione (GSH), the principal scavenger of reactive oxygen/nitrogen species, which results in the depletion of GSH, intracellular free radical formation and tumor cell death [1,4].

In the previous preclinical studies and Phase I-II clinical trials, intravenous administration of RRx-001 has demonstrated tumor growth inhibition and prolonged survival in patients [1-2,5]. Preclinically RRx-001 has been found to protect normal tissues such as intestinal crypt stem cells, bone marrow and skin tissues against chemotherapy- and ionizing irradiation-induced damage and to promote survival [2,6]. RRx-001 activates nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant signaling pathways [7], suggesting a mechanism for its potential protective effects. In clinical trials, RRx-001 is minimally toxic with non-dangerous, transient infusion related discomfort as the chief adverse event [5].

Oral dosing is a convenient and safe method for administration of anticancer agents. Herein are reported the results of preclinical pilot studies, which demonstrate that oral administration of RRx-001 both as a single agent monotherapy and as a combination therapy with radiotherapy is safe and effective.

Materials and Methods

Animals

C3H mice, male, 7-8 weeks old and 20-25 grams in body weight were purchased from Taconic Biosciences. Mice were housed in the Veterinary Service Center (VSC) of Stanford University animal facilities. Mice were acclimated for 3-5 days before starting the experiment. All animal experiments were approved by and complied with the regulations of the Stanford University Animal Care Panel.

Oral administration of RRx-001

RRx-001 was obtained from ATK Aerospace Systems RRx-001 solution was prepared freshly and used on the same day. RRx-001 was dissolved in DMSO and then diluted with double distilled water to obtain a final solution of 2-6 mg/mL. The final DMSO concentration was 5-10%. RRx-001 was administered orally in a volume of 5-10 μL/gram body weight.

For oral administration of RRx-001, mice were firmly held in an upright position and the neck and head are immobilized. The head was slightly tilted back towards the spine with gentle pressure from the gavage needle. The gavage needle was slid into the animal mouth between the tongue and the front teeth and slid down further into the esophagus. The RRx-001 solution was slowly injected. After oral gavage, mice were monitored for at least 10 minutes and observed for potential complications or abnormal reactions.

Radiation Therapy

Radiation therapy was delivered using a Polaris SC-500 250kVp X-ray machine (12.5 mA; half value layer, 0.5-mm Cu). For local tumor irradiation, unanesthetized mice were placed in individual lead jigs with tumors protruding through a cut-out window at the rear of each jig and irradiated locally to the tumors with 250 cGy per fraction per day for 5 consecutive days at a dose rate of 2 Gy/min.

Tumor Model

Mice were inoculated subcutaneously on the back with 5x105 squamous carcinoma SCC VII tumor cells in 0.05 ml Hank's solution. Ten (10) days after tumor implantation, mice with tumors ranging from 150-250 mm3 in size were randomized into treatment groups. There was no statistically significant difference in tumor size and animal body weight among groups on the first day of treatment (Day 0). The length and width of the tumors were measured with calipers immediately before treatment and three times a week thereafter until the tumor volume reached at least four times (4X) the original pre-treatment volume. Tumor volume (mm3) was calculated according to the formula: tumor volume = $\pi/6$ x length x width2. The data are expressed as percentage of pre-treatment volume measured on Day 0 as a function of days from start of the treatment. The tumor volume quadrupling time (4X TGT, in days) was determined by a logarithmic regression analysis. The tumor growth delay (TGD) time is the difference between the 4X TGT of treated tumors compared to that of untreated vehicle control tumors. Both the 4X TGT and TGD time was calculated for each individual animal, and then averaged for each group.

Result

MTD of oral administration of RRx-001 in mice

Normal C3H mice were treated with RRx-001 administered by oral gavage with RRx-001 at doses ranging from 10-80 mg/kg, daily for 5 consecutive days. Following oral administration, mice were monitored for mortality, physical activity and body weight. Results showed that a daily dose of 10 mg/kg was well tolerated and caused no mortality. The highest daily dose of 80 mg/kg caused 100% mortality (4 death of 4 mice total). The maximum tolerated dose for daily oral administration for 5 days was estimated at 10-20 mg/kg. The average body weight of mice after oral dosing of 20-60 mg/kg RRx-001was decreased by 7-10% of pretreatment body weight between Days 2-9.

Antitumor efficacy of oral administration of RRx-001

The therapeutic efficacy of oral administration of RRx-001 in an immunocompetent syngeneic model of squamous cell carcinoma SCC VII was studied. Tumor-bearing mice were randomized into four groups and treated with oral RRx-001 at a total equivalent dose of 60 mg/kg. The treatment arms are Group 1: 12 mg/kg, daily for 5 days on Day 0-4; Group 2: 20 mg/kg, every other day for 3 doses on Day 0, 2, and 4; Group 3: 60 mg/kg, one single dose on Day 0; and Group 4: 10% DMSO daily for 5 days without oral RRx-001. Results are shown in Table 1A and Figure 1A. Oral administration of RRx-001 at these doses significantly inhibited tumor growth ($p < 0.01$ vs. vehicle control) and produced a tumor growth delay time of 1.3 -1.5 days. There was no statistically significant difference in tumor growth delay time among three RRx-001 treated groups. However, there were two mice that died in Group 3 with a single dose treatment of 60 mg/kg.

Antitumor efficacy of RRx-001: oral vs. intravenous administration

Groups of mice with established SCC VII tumors were treated with either oral gavage or intravenous injection of 10 mg/kg RRx-001 every other day for 3 doses total from Days 0-4. A control group of mice with tumors received 10% DMSO orally. Seven animals were used in each group. Results showed that both regimens of oral and intravenous dosing of RRx-001 inhibited tumor growth and produced the tumor growth delay times of 1.2 ± 0.4 days and 3.0 ± 1.4 days for oral and intravenous dosing, respectively ($p < 0.01$ compared with Control group). However, at the same dose level, intravenous dosing was more effective in inhibition of tumor growth than oral dosing in terms of the tumor growth delay time ($p < 0.05$). Both oral and intravenous administration of RRx-001 were well tolerated.

Combination therapy of oral RRx-001 and radiotherapy

Mice bearing SCC VII tumors were treated with an oral dose of 10 mg/kg RRx-001 or 250 cGy local tumor radiation, either alone or in combination daily for 5 consecutive days. As shown in Table 1B and Figures 1B, oral dosing with 10 mg/kg RRx-001 produced a moderate inhibition of tumor growth ($P = 0.01$ vs. Control). Radiation therapy alone inhibited tumor growth and produced a tumor growth delay time of 0.9 ± 0.7 days ($P = 0.01$ vs. Control). When the use of oral RRx-001 and radiation were combined, the tumor growth delay time was significantly increased from 0.5–0.9 days for either modality alone to 3.1 ± 1.8 days for RRx-001 plus radiation ($P < 0.01$ vs. Control). More importantly, oral RRx-001 did not increase the systemic toxicities of radiotherapy as measured by the body weight change of mice (Figure 1C).

Toxicity of oral RRx-001

A daily oral dose of 10 mg/kg for 5 days was well tolerated. Cumulative toxicity was not observed. Following oral administration, there were no unexpected deaths, and no obvious change in the general appearance, skin reaction, or daily activity of mice. There were no signs of severe systemic toxicities following oral dose of 10 mg/kg daily for 5 days.

Discussion

These experiments demonstrate that orally administered RRx-001 is safe and inhibits tumor growth in tumor-bearing mice. Evidence of anticancer activity with RRx-001 has already been demonstrated clinically and preclinically via the intravenous route [1-2,5]. The reason to study the effects of oral administration is two-fold: 1) to circumvent the infusion related reactions (chiefly discomfort) associated with RRx-001 and 2) for the purpose of radiation protection. In the chaotic aftermath of a radiation accident or radiological terrorism, protection of emergency responders, military personnel and the at-risk civilian population with an agent like RRx-001 requires oral or subcutaneous delivery rather than intravenous administration, which is highly impractical and inconvenient. In addition, since acute radiation syndrome (ARS) occurs after whole-body or significant partial-body irradiation and involves the gastrointestinal tract, oral administration may preferentially protect the intestinal epithelium. In general, the disadvantage of oral administration is poor absorption and/or bioavailability. In the case of RRx-001, these experiments demonstrate that orally administered RRx-001 in mice is bioavailable, well tolerated and active. Future studies will further assess

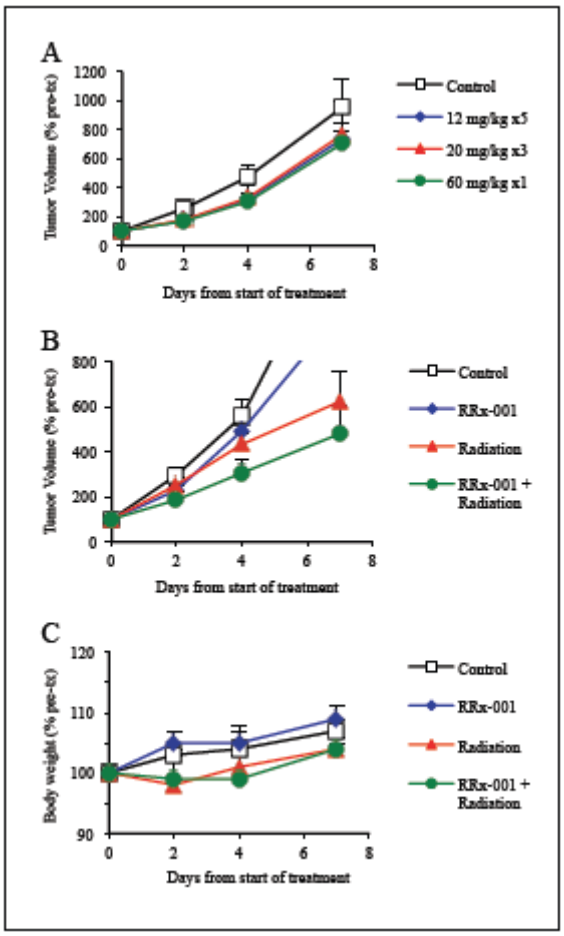

Figure 1: A. Tumor growth curves in mice treated with oral RRX-001 alone. B. Tumor growth curves in mice treated with combination of oral RRx-001 and local tumor irradiation. C. Body weight of tumor-bearing mice treated with combination of oral RRx-001 and local tumor irradiation.

Table 1: Tumor growth delay time of SCC VII tumors in mice
A. Oral RRx-001 alone

	Number of mice	Number of death	4X TGT (day)	TGD (day)	P value (*t*-test)		
					Control	12mg/kg	20mg/kg
Untreated Control	7		3.5 ± 0.6				
12 mg/kg, QD x 5	6		4.9 ± 0.4	1.3 ± 0.4	<0.01		
20 mg/kg, QOD x 3	7		4.9 ± 1.0	1.3 ± 1.0	0.01	1.0	
60 mg/kg, one dose	7	2	5.0 ± 0.3	1.5 ± 0.3	<0.01	0.5	0.7

	Number of mice	4X TGT (day)	TGD (day)	P value (*t*-test)		
				Control	RRx-001	Radiation
Untreated Control	7	3.0 ± 0.3				
RRx-001 10mg/kg QD x 5	7	3.5 ± 0.4	0.5 ± 0.4	0.01		
Radiation 250cGy daily x 5	7	3.9 ± 0.7	0.9 ± 0.7	0.01	0.3	
RRx-001 + Radiation	7	6.1 ± 1.8	3.1 ± 1.8	<0.01	0.01	0.02

B. Combination of RRx-001 and radiation

pharmacokinetics, bioavailability, food interaction and toxicity.

Conclusion

The purpose of this pilot study was to evaluate the tolerability and antitumor efficacy of oral administration of RRx-001 both alone and in combination with radiation therapy in mice. All available clinical data with RRx-001 are based on intravenous therapy; however, oral administration is a more practical alternative. Oral administration of RRx-001 at its MTD dose daily was well tolerated and significantly inhibited tumor growth. When combined with radiation therapy, oral RRx-001 increased the antitumor efficacy of radiation therapy without increasing the radiation-induced systemic toxicities. Hence, RRx-001 can be safely administered orally in mice.

In the quest to find minimally toxic, orally bioavailable 'dual function' agents that selectively sensitize tumors to radiotherapy and also preferentially protect normal tissues, RRx-001 is potentially an ideal candidate. However, further work is required to determine the suitability of RRx-001 for oral formulation development.

Compliance with ethical standards

Conflict of interest

B. Oronsky, J. Scicinski and C.A. Carter are employees of Epicent Rx, and S.J. Knox is a founder of Epicent Rx. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Ethical statement

All animal experiments were approved by and complied with the regulations of the Stanford University Animal Care Panel.

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