RRx-001 Oxidation of Redox Sensitive Protein Thiols in Tumors Measured by Gd-LC7-SH Enhanced MRI in Preclinical Tumor Models

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ABSTRACT

Permanent Abstract Number 2068. RRx-001 is a member of the novel dinitroazetidine-containing class of anticancer agents that profoundly perturbs the thiol redox potential of the cancer cell while subjecting it to damaging ROS/RNS and acting as an epigenetic modifier. A multi center Phase 1 study investigating the safety and tolerability of RRx-001 has been completed and Phase 2 studies are ongoing. Pre-clinical studies indicate that RRx-001 selectively alkylates glutathione and a specific thiol on hemoglobin, resulting in a pro-oxidant effect in tumors [1, 2]. We have previously reported the development of thiol-containing DOTA-based chelates of gadolinium as redox-sensitive MRI contrast agents [3, 4]. We now present a preclinical MRI investigation of the pharmacodynamics and mechanism of action of RRx-001 using a novel thiol-bearing contrast agent, Gd-LC7-SH.

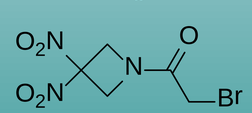
Severe Combined Immunodeficient (SCID) mice were inoculated in the flank with either CHP-100 Ewing's Sarcoma, HT-29 colorectal carcinoma, or PANC-1 pancreatic carcinoma cells. Mice were imaged on a 7 Tesla Bruker Biospec® small animal MRI scanner when tumors had grown to 250-400 mm3 in size. Mice were anesthetized using isoflurane (1.5-2.5% in O_2, 1 L/min) and cannulated at the tail vein for injections using a "zero dead volume" i.v. line (~25 μL dead volume) for administration of Gd-LC7-SH or RRx-001. Longitudinal relaxation time (T1) maps of the tumor were acquired pre-contrast and at various times post-contrast to 60 min post-injection of 0.05 mmol/Kg Gd-LC7-SH. Mice were imaged before treatment and at 1h, 24 h and 72 h post-treatment with 10 mg/Kg RRx-001.

Gd-LC7-SH spontaneously binds to thiol targets following i.v. administration. The fraction of gadolinium that is bound to macromolecular targets such as plasma albumin and exofacial protein thiols (EPTs) is protected from renal clearance, and produces a prolonged decrease in tumor T1 on MRI. We have quantified this decrease in tumor T1 by the parameter ΔT1, calculated as pre-contrast tumor T1 minus tumor T1 at 40-60 min post Gd-LC7-SH. The larger the magnitude of ΔT1, the greater is the retention of Gd-LC7-SH in the tumor. In all 3 tumor types, the tumor ΔT1 at 1 h post-drug was significantly smaller than pre-drug tumor ΔT1 (p<0.02). In the HT-29 and PANC-1 tumors the ΔT1 at 72 h post-drug remained smaller than baseline ΔT1 (p<0.05). These observations indicate decreased tumor retention of Gd-LC7-SH following treatment with RRx-001, which is consistent with a decrease in availability of reduced albumin and EPTs in the tumor.

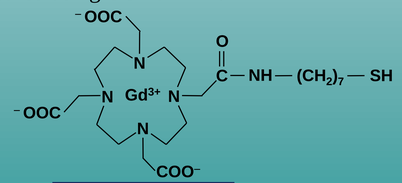
The previously reported redox activity of RRx-001 together with its very short half-life in vivo suggests an indirect effect on albumin and exofacial thiols that manifests as smaller ΔT1 values on Gd-LC7-SH MRI imaging. The anti-proliferative activity of RRx-001 may not only be due to glutathione depletion and NO release under hypoxia, but also to an increase in intratumoral ROS burden leading to a direct redox modulation of exofacial thiols integral to tumor protein function. Additional studies are planned to confirm this postulate.

OBJECTIVES

The primary objective of this study was to investigate the mechanism of action and pharmacodynamics of RRx-001 using thiol-sensitive MRI enhanced by Gd-LC7-SH. We have hypothesized that alkylation of thiols in the tumor by RRx-001 will manifest as measurable differences in the post-contrast change of tumor T1 between control and treated animals. Additionally, we have hypothesized that RRx-001 alkylation of a specific thiol on hemoglobin can be measured using BOLD MRI.

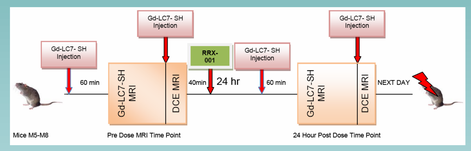


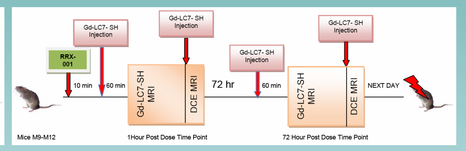
RRx-001



Gd-LC7-SH

METHODS

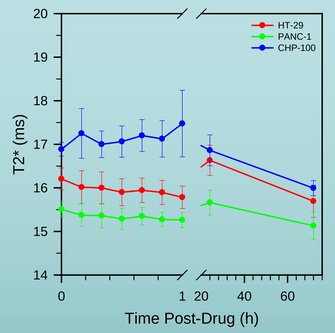


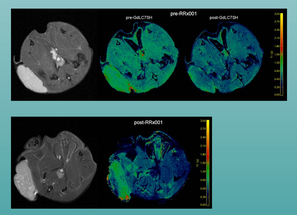


SCID mice were inoculated in the flank with either CHP-100 Ewing's Sarcoma, HT-29 colorectal carcinoma, or PANC-1 pancreatic carcinoma cells. MRI was performed when tumors were 250-400 mm^3 in volume. Mice were imaged before treatment and at 1h, 24 h and 72 h post-treatment with 10 mg/Kg RRx-001 (*i.v.*). T1 maps were acquired pre-contrast and at various times post-contrast to 60 min post-injection of 0.05 mmol/Kg Gd-LC7-SH (*i.v.*) as per the schedule depicted above. T2* maps were acquired by BOLD MRI on a separate cohort of mice as per an analogous schedule.

RESULTS

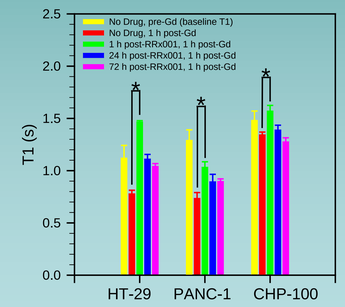
BOLD MRI (right). Variation of tumor T2* (mean ± S.E.M., *n*=4) before *vs.* following treatment with RRx-001 (10 mg/Kg, *i.v.*). RRx-001 is known to alkylate a thiol on hemoglobin (Hb). Drug action did not result in a significant change in T2* of any of the 3 tumor models studied.





Gd-LC7-SH MRI (left). Before treatment with RRx-001 (top panel) the T1 of both tumor and muscle are significantly lower 60 min post-Gd-LC7-SH. After RRx-001 exposure (bottom panel) the T1 of both tumor and muscle are higher than expected 60 min post-Gd-LC7-SH, consistent with a rapid clearance of Gd-LC7-SH due to oxidation of exofacial protein thiols & albumin in the tumor.

RESULTS (contd.)



Gd-LC7-SH MRI (left). Variation of tumor T1 (mean ± S.E.M., *n*=4) before *vs.* following treatment with RRx-001 (10 mg/Kg, *i.v.*). In all 3 tumors, Gd-LC7-SH produced a prolonged decrease in tumor T1 evident even at 60 min post-injection (red *vs.* yellow). This decrease was abolished or greatly decreased 1 h post-treatment with RRx-001 (green *vs.* red) in all 3 tumors. In HT-29 and PANC-1 tumors this effect of RRx-001 on post-GdLC7SH tumor T1 was apparent even at 72 h post-drug (blue & pink *vs.* red).

CONCLUSIONS

BOLD MRI results suggest that RRx-001 action does not produce a change in oxidation state of the Fe in hemoglobin and/or result in significant iron deposition in the tumor tissue, either of which would have manifested as a perturbation of tumor T2*. Although redox-sensitive MRI supports alkylation of thiols by RRx-001 as a mechanism of action, the known redox activity of RRx-001 together with its very short half-life *in vivo* strongly suggest an indirect drug effect on albumin and exofacial thiols in the tumor that manifests as smaller ΔT1 values on Gd-LC7-SH MRI imaging. These effects could be attributed to the oxidation or nitrosylation of tumor exofacial thiols by ROS and RNS derived from RRx-001. The anti-proliferative activity of RRx-001 may not only be due to glutathione depletion and NO release under hypoxia, but also to an increase in intratumoral ROS burden leading to a direct redox modulation of exofacial thiols integral to tumor protein function.

REFERENCES

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