Measurements by EPR and BOLD of the Effectiveness of an Allosteric Hemoglobin Effector, RSR13, to Repetitively Enhance Tumor Oxygenation

H. Hou[1], N. Khan[1], J. A. O'Hara[1], O. Y. Grinberg[1], J. F. Dunn[1], M. A. Abajian[1], E. Demidenko[1], R. P. Steffen[2], H. M. Swartz[1]

[1]Dartmouth Medical School, Hanover, NH, United States, [2]Allos Therapeutics, Inc., Westminster, CO, United States

SYNOPSIS

Using *in vivo* EPR oximetry and BOLD imaging, the time course of the effect of RSR13, on tumor pO_2 was measured. RSR13, (2-(4-(2-[(3,5-dimethylphenyl) amino]-2-oxoethyl]phenoxy]-2-methylpropanoic acid) has been shown to enhance tissue oxygenation by decreasing the affinity of hemoglobin for oxygen thus promoting release of oxygen from erythrocytes to tissue. The results show that daily RSR13 treatment significantly and repetitively enhanced oxygenation of the tumor (RIF-1 in the mouse) in a range that would effectively increase the cytotoxicity of ionizing radiation to the tumor. The results also illustrate the potential power of EPR and BOLD to provide data on tumor oxygenation that would be very difficult to obtain by any other method.

METHODS

The study was carried out in transplanted RIF-1 tumors in 14 female C3H/HEJ mice, and Lithium Phthalocyanine (LiPc) implanted in the tumor when the tumors reached about 1 cm^3. Baseline measurements of tumor pO_2 were made for three days. Starting on the fourth day of EPR measurements, after an initial baseline measurement – RSR13 (150 mg/kg) was injected intraperitoneally and measurements of intratumoral oxygen were made for 60 minutes following treatment. The administration of RSR13 was carried out for six consecutive days. In each mouse, every third day, BOLD NMR measurements were made instead of EPR oximetry for the 60-minute period following RSR13**.** The EPR measurements were made with a 1200 GHz *in vivo* spectrometer developed and built at the EPR Center for the Study of Viable Systems. The BOLD NMR measurements were done at 7T using a multi-echo gradient echo sequence with TR=1.5s, TE=0.007s and an interecho spacing of 0.005s. SI vs TE the data were fit to a single exponential function to quantify R2*. After the measurements on the sixth day of administration of RSR13, the animals were sacrificed and studied to determine the histological status of the tumor, including the position of the LiPc within the tumor.

RESULTS

As indicated in the table, RSR13 resulted in a significant increase of the tumor pO_2 at most time points over the sixty-minute period. There were statistically significant changes in the magnitude of the effect of RSR13 over the 60 minute time course, with a maximum reached at 30-40 minutes. RSR13 increased R2* significantly ($p=0.006$) $\Delta R_2^*=2.5\pm0.8\ s^{-1}$ (30 min post-infusion – pre-infusion, mean±SE). The increase in dHb (indicated by the increase in R2*) is consistent with an increase in total dHb. Since Amorino et al. showed a decrease in R2* with RSR13 administration, these data indicate that relation of BOLD with alterations of tumor oxygenation by RSR13 may be tumor specific. This indicates the value of direct measurements of pO_2 in tumors to determine changes in oxygenation.

DISCUSSION

The magnitude of the observed increase of oxygen in the tumor has significant implications for enhancing the effectiveness of radiation therapy. The measured increase from the baseline of tumor pO_2, would be expected to lead to a significant increase in the tumor radiosensitivity. These results illustrate a unique and useful capability of EPR oximetry, obtaining repetitive measurements of tissue (tumor) pO_2 noninvasively. This provides unambiguous information on the effectiveness of RSR13 to repetitively enhance tumor oxygenation.

Table 1. Mean baseline pO_2 and mean changes of tumor pO_2 (ΔpO_2) after daily treatment of RIF-tumors with RSR in mice

	CHANGES IN pO_2 FROM BASELINE AFTER RSR13 AT 10 - 60 MINUTES						
Time	BASELINE	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
[days]	pO_2						
Day 1	**5.71+/- 1.16**	3.72+/-3.37	8.16+/-3.15*	9.39+/-2.80*	6.93+/-2.96*	4.79+/-4.07	5.07+/-3.05
Day 2	**7.19+/-1.24**	4.90+/-2.68	13.26+/-4.63*	15.56+/-4.74*	8.82+4.14	8.36+/-3.44*	12.57+/-7.07
Day 3	**4.73+/-0.76**	0.65+/-0.7	1.79+/-0.69*	6.51+/-1.82**	6.46+/-2.39*	3.00+/-1.24*	1.06+/-1.28
Day 4	**5.24+/-0.46**	(-)0.45+/-0.54	1.37+/-0.83	3.99+/-1.60*	10.40+/-4.47*	7.89+/-2.79*	5.99+/-2.54
Day 5	**10.43+/-3.23**	(-)0.26+/-1.77	1.22+/-3.46	4.34+/-2.96	5.02+/-3.55	4.14+/-2.92	3.58+/-2.17
Day 6	**3.99+/-0.77**	0.45+/-0.61	2.59+/-1.56	3.44+/-1.52*	1.57+/-0.67*	1.65+/-1.09	0.78+/-0.30*

The baseline is the average pO_2 from the 30 min period prior to the injection of RSR-13. Numbers are the change in tumor pO_2 (mm Hg) from baseline (changes are increases in pO_2 except where noted as (-)). Results are expressed as mean +/- SEM (N = 14 at minutes 10 to 30, N = 10-14 at minutes 40 to 60). * $p< 0.05$; ** $p < 0.01$, compared with baseline, two-tailed paired t-test.

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