Hyperpolarized MRSI

Mapping metabolic changes associated with early Radiation Induced Lung Injury post conformal radiotherapy using hyperpolarized $^{13}$C-pyruvate Magnetic Resonance Spectroscopic Imaging

Kundan Thind$^{a,b,*}$, Michael D. Jensen$^a$, Elaine Hegarty$^b$, Albert P. Chen$^c$, Heeseung Lim$^{a,b}$, Francisco Martinez-Santiesteban$^{b,e}$, Jake Van Dyk$^{a,e}$, Eugene Wong$^{a,b,d}$, Timothy J. Scholl$^b$, Giles E. Santyr$^{a,b,f}$

$^a$Dept. of Medical Biophysics; $^b$Imaging Research Laboratories, Robarts Research Institute, Western University, London, Canada; $^c$GE Healthcare, Toronto; $^d$Dept. of Physics and Astronomy; $^e$Imaging Research Laboratories, Robarts Research Institute, Western University, London, Canada; $^f$Dept. of Oncology; and $^g$Dept. of Medical Imaging, Western University, London, Canada

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ABSTRACT

Purpose: Radiation Pneumonitis (RP) limits radiotherapy. Detection of early metabolic changes in the lungs associated with RP may provide an opportunity to adjust treatment before substantial toxicities occur. In this work, regional lactate-to-pyruvate signal ratio (lac/pyr) was quantified in rat lungs and heart following administration of hyperpolarized $^{13}$C-pyruvate magnetic resonance imaging (MRI) at day 5, 10, 15 and 25-post conformal radiotherapy. These results were also compared to histology and blood analyses.

Methods: The lower right lungs of 12 Sprague Dawley rats were irradiated in 2 fractions with a total dose of 18.5 Gy using a modified micro-CT system. Regional lactate and pyruvate data were acquired from three irradiated and three age-matched healthy rats at each time point on days 5, 10, 15 and 25-post radiotherapy. Arterial blood was collected from each animal prior to the $^{13}$C-pyruvate injection and was analyzed for blood lactate concentration and arterial oxygen concentration ($p_O_2$). Macrophage count was computed from the histology of all rat lungs.

Results: A significant increase in lac/pyr was observed in both right and left lungs of the irradiated cohort compared to the healthy cohort for all time points. No increase in lac/pyr was observed in the hearts of the irradiated cohort compared to the hearts of the healthy cohorts. Blood lactate concentration and $p_O_2$ did not show a significant change between the irradiated and the healthy cohorts. Macrophage count in both right and left lungs was elevated for the irradiated cohort compared to the healthy cohort.

Conclusions: Metabolic changes associated with RP may be mapped as early as five days post conformal radiotherapy. Over the small sample size in each cohort, elevated macrophage count, consistent with early phase of inflammation was highly correlated to increases in lac/pyr in both the irradiated and unirradiated lungs. Further experiments with larger sample size may improve the confidence of this finding.

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Lung cancer continues to be responsible for the highest number of cancer related deaths [1,2]. Radiotherapy is a primary treatment method for lung cancer [3] but limited by adverse consequences due to the high radio-sensitivity of the lung [4]. A major side effect of thoracic radiotherapy is Radiation Pneumonitis (RP) and can proceed to irreversible lung fibrosis in the later stages [3]. RP occurs in up to 37% of lung cancer patients receiving radiotherapy [3,5], with moderate to severe RP occurring in about 20% of patients [6].

Early detection of RP using imaging could potentially improve patient outcome by appropriate modification of the radiotherapy treatment plan, dose-reduction in chemotherapy and/or administration of corticosteroids to help reduce inflammation [7]. Chest X-ray and Computed Tomography (CT) imaging are the most commonly used methods for detection of Radiation-Induced Lung Injury (RILI) in the clinic. These techniques detect changes in lung density and can be useful for the evaluation of fibrosis associated with late stage injuries [8]. Three-dimensional (3D) CT has been used to provide lung dose–volume histogram (DVH) parameters to forecast...

* Corresponding author. Address: Robarts Research Institute, Western University, 1151 Richmond St. N., London, Ontario N6A 5B7, Canada.
E-mail address: kundanthind@gmail.com (K. Thind).
the development of RP [9]; however, these parameters have very low predictive powers [3]. Nonetheless, some of the DVH parameters are strongly correlated with the incidence of RP [10]. Recently, Positron Emission Tomography has shown increased uptake of 18F-Fluorodeoxyglucose associated with RP in lung cancer patients [7].

Despite the low proton signal afforded in the lung, Magnetic Resonance Imaging (MRI) has been used for the detection of RP, including Dynamic contrast-enhanced MRI [11] and T₁- and T₂-weighted contrast MRI [12]. Recently, hyperpolarized 3He-MRI has been used to detect RP post irradiation in lung cancer patients [13]. However, all these techniques are based on morphological and functional changes, which may occur in the later stages of injury. Imaging biomarkers sensitive to early metabolic changes associated with RP at the cellular level could provide an approach for earlier detection of RILI.

At a cellular level, immunohistochemical analysis of RP in animal models reveals underlying changes in DNA and increases in alveolar macrophages, inflammatory cytokines and type II pneumocytes [14,15]. These changes are observed in both in-field and out-of-field post conformal radiotherapy [16,17], and persist up to 4 weeks post irradiation. [18] Activation of T-lymphocytes has also been observed as a protection mechanism against radiation fibrosis [19]. The occurrence of RP is closely tied to the onset of inflammation, which is highly dependent on the onset of inflammatory cytokines. [20] Additionally, hypoxia in lung tissue plays a role in the mediation of inflammatory process in RP [21]. Decreased arterial partial pressure of oxygen (pO₂), which is used as an indicator of hypoxia [13] has been reported following radiotherapy [22,23]. Recent observations from lung cancer patients post irradiation suggest a strong correlation between dynamic changes in CD35 and onset of RP [24]; and between the amount of exhaled NO and occurrence of RP [25].

Advances in Dynamic Nuclear Polarization (DNP) technique have enabled the in vivo MRI of carbon-13 (13C) substrates [26]. The pyruvate molecule enriched with 13C at the C-1 position has been used extensively as it can be polarized to a high degree and the conversion of 13C-pyruvate to its metabolic products, 13C-lactate, 13C-bicarbonate and 13C-alanine, has been readily observed in vivo [27,28]. Specifically, changes in 13C-lactate to 13C-pyruvate signal ratio (lac/pyr) have been used to measure lung ischemia ex vivo [20]. Our research has previously demonstrated changes in lac/pyr in a rat model of RILI at two weeks post whole thorax irradiation [29].

The purpose of this work was to quantify regional lac/pyr using hyperpolarized 13C Magnetic Resonance Spectroscopic Imaging (MRSI) in a rat model of RP involving conformal radiotherapy of the lung, 5, 10, 15 and 25 days post-irradiation. These results were compared to those obtained in healthy age-matched rats and correlated with quantitative (i.e. cell counting) histological analysis of the lung following imaging. The potential origin of lac/pyr changes in RP is discussed in terms of increases in macrophage population.

**Methods**

**Animal irradiation**

All procedures followed animal care protocols approved by the Western University (ACVS) and were consistent with procedures used by the Canadian Council on Animal Care (CCAC). The right mediastinal lobe of the lungs of 12 Sprague Dawley rats (200 ± 50 g) was irradiated with a total dose of 18.5 Gy (2 × 9.25 Gy, 24 h apart, respiratory-gated delivery) using a modified micro-CT system (GE eXplore CT 120, GEHC, Waukesha, WI) system [30]. Fig. 1 shows a representative conformal dose distribution in the lungs and heart region of rat thorax for a single fraction (details provided in supplementary material). A total of 12 irradiated and 12 age-matched healthy rats were divided equally into four groups. One group was imaged and sacrificed per time point (5, 10, 15 and 25 days post-irradiation) for MRI and histology data.

**Blood lactate, pO₂ measurements and histology preparation**

To quantify the systemic spread of disease, blood lactate and pO₂ readings were acquired from each animal pre-MRI. Lung histology was obtained from each animal post-MRI to quantify the macrophage count (details provided in supplementary material).

**Magnetic resonance spectroscopy and imaging**

Hyperpolarized 13C-pyruvate was prepared and administered as previously described [29]. MRI and MRSI were also performed as previously described [29] using a 3T MR imaging system (MR750, GEHC, Waukesha, WI). For each cohort, rats were randomly chosen and the measurements were performed in a blinded fashion. To receive 13C-MRSI signal, a custom 13C-surface coil was wrapped around the animal thorax. The tray was then inserted into a custom-built, rat-sized quadrature dual-tuned (13H–13C) birdcage coil, which was used to transmit/receive signal for 13H-MRI and to transmit-only for 13C-MRSI.

**1H MRI**

T₁-weighted 1H images of the rat thorax were acquired in the coronal and axial planes with parameters described previously [29]. These images were used to localize the 13C-MRSI volume of interest within the lungs, as the lung boundary was well visualized as a signal void compared to the other tissues (e.g. heart and liver).

**13C-MRSI**

MRSI was performed using a Chemical Shift Imaging (CSI) technique as described previously [29]. A slab was placed above the diaphragm with a margin sufficient to minimize respiratory motion related contamination of the heart and the lung signal with liver signal. The slab was centered on the lungs and divided into a 12 × 12 matrix with minimal coverage of the liver and the heart. Spectroscopic imaging was initiated following a delay of 25 s from the start of the bolus injection to maximize the lactate signal [29].

**13C MRSI data processing and analysis**

SAGE™ software (GEHC, Waukesha, WI) was used for processing of the acquired data. The 13C-MRSI data were apodized with a 10 Hz Gaussian filter in the time domain, zero-filled 1 in the time dimension and both spatial dimensions. Fourier transformations were then performed in both the time domain and the two spatial dimensions to obtain a 16 × 16 grid of spectra with each voxel of dimensions: 3.75 mm × 3.75 mm × 8 mm. The metabolite peaks were phased and baseline corrected for every voxel. The 16 × 16 grid of spectra was overlaid on the axial 1H image and twelve voxels were chosen: four centered on the left lung, four on the right lung and four on the heart. The relative positions of these four voxels for each organ were kept consistent for each animal. The measure of lac/pyr for each group of four voxels was computed by dividing the total lactate signal intensity by the total pyruvate signal intensity. Two-tailed paired T-tests were performed (with the assumption of unequal variance) for the blood lactate concentration level, pO₂ level, lac/pyr measurements from the left lung, the right lung and the heart between the irradiated cohort and the healthy cohort for each time point. A p-value of less than 0.05 was considered significant. A mixed analysis of variance
Results

Fig. 2 shows a typical CSI grid overlaid on the corresponding 1H image for a representative irradiated rat, including the voxels chosen for analysis of lac/pyr in the left lung, right lung and heart region respectively. Fig. 3 provides a summary of the average lac/pyr measurements obtained from these regions in both the irradiated and healthy cohorts at day 5, 10, 15 and 25-post irradiation. Both right and left lungs from the irradiated cohort show a significant increase in lac/pyr compared to the right and left lung from the healthy cohort respectively at each time point (p < 0.05). This confirms the response to radiation both at the site of the irradiation (medial right lung) as well as the unirradiated (i.e. contralateral) left lung.

Mixed analysis of variance demonstrated a significant decrease in lac/pyr at day 25 compared to day 10 in the right lung of the irradiated cohort (p < 0.05), suggesting the RP has begun to subside. Table 1 summarizes lac/pyr (mean ± first standard deviation) from the right lung, left lung and heart for the irradiated and healthy cohorts for all time points.

Measured lac/pyr was not significantly different in the heart region of the irradiated cohort compared to the heart region of the healthy cohort at any time point post irradiation (p > 0.05). At day 25, lac/pyr was lower in the heart region of the irradiated cohort compared to the heart region of the healthy cohort (0.21 ± 0.01 vs. 0.25 ± 0.01, n = 3, p < 0.05). Analysis of the histology from the heart region at day 10 and day 15-post irradiation confirmed the absence of injury.

Investigation of blood lactate concentration levels did not show significant changes between the irradiated and healthy cohorts at any time point (p > 0.05) post irradiation with non-time point specific measurements being 1.22 ± 0.3 vs. 1.23 ± 0.31, n = 12, respectively. Additionally, blood pO2 level measurements did not show significant changes between the irradiated and healthy cohorts at any time point (p > 0.05) and pO2 did not drop below normal (60 mmHg) for any of the irradiated cohorts. Measurement from all irradiated and healthy cohorts (non-time point specific) were 80 ± 15 vs. 78 ± 14, n = 12, respectively. The conformal irradiation of the right lung does not appear to compromise the pO2 and might be explained by a compensatory effect of the unirradiated lung, enabling adequate oxygen supply to the body. The heart tissue is also substantially spared injury due to the conformal nature of the irradiation, therefore not compromising cardiac function. In addition to the conformal radiotherapy, the lower dose rate of the radiation beam used in this study may have reduced the level of tissue injury compared to our previous study [29].
black arrows. Table 1 summarizes the macrophage count for the right and left lung from irradiated and healthy cohorts for each time point. Fig. 4 shows the time courses for average lac/pyr and macrophage count and a scatter plot of the lac/pyr measurements versus macrophage count for the right and left lung of all the rats for all time points. Macrophage counts were observed to increase in both the right and left lungs of the irradiated cohort compared to the healthy cohort, following a trend similar to lac/pyr at each time point. Non-parametric analysis of these data provided Spearman coefficients (r) of 0.86 (p < 0.01) and 0.85 (p < 0.01) respectively for the right and left lung.

Discussion

In this rat model of RP involving conformal irradiation of the right lung, measures of lac/pyr were shown to be elevated in both lungs at all time points following irradiation. This is consistent with previous observations in a similar rat model, which showed that cytokines and DNA damage persist 5–15 days and 3 days to 4 weeks post irradiation, respectively [18]. Significant decreases in lac/pyr in the right irradiated lung observed at day 25 compared to day 10 may be evidence of reduced inflammation from the receding initial phase of RP. This seems to be supported by the reduction in measured macrophage activity at the later time points (Fig. 4).

Additionally, lac/pyr increases confirm the response of the whole lungs to conformal radiotherapy, both at the site of irradiation (medial right lung) and at the unirradiated contralateral side (left lung). This observation is in agreement with previous findings, which have demonstrated that conformal radiotherapy of the lungs results in injury response of the lungs both in and out of the radiation field [16,17].

Measurements of lac/pyr did not show altered heart metabolism at any time point. This was confirmed by the analysis of the histology from the heart region. Lack of increase in lac/pyr in the heart region, increase in blood lactate levels and decrease in $p_{O_2}$ in the heart region, increase in blood lactate levels and decrease in $p_{O_2}$
in irradiated cohorts compared to healthy cohorts demonstrates that the injury response was limited to the lungs with no systemic effects. This confirms that the irradiation conformed to the lungs, sparing other surrounding tissues.

Macrophage count in both the right and left lungs of the irradiated cohorts was higher than the corresponding right and left lung of healthy cohorts, which is in agreement with previous observations showing that macrophages are recruited from outside the

Fig. 4. Time evolution of lac/pyr in the healthy and irradiated cohorts in the right lung shown in (a) and in the healthy and irradiated cohorts in the left lung shown in (b). Sub-plots (c) and (d) show progression of macrophage count in the healthy and irradiated cohorts in the right and left lung, respectively. Sub-plots (e) and (f) show scatter of lac/pyr and macrophage count and the Spearman correlation coefficient in the right lung ($r = 0.86$, $p < 0.01$) and the left lung ($r = 0.85$, $p < 0.01$), respectively (mean ± 1 SD).
lungs [31] and that these infiltrating macrophages are a source of cytokines associated with onset of inflammation in early RP [32]. As such, the quantitative measure of macrophages is a reliable indicator of the inflammation in early RP. The hypothesis that lac/pyr changes may be a reflection of increased macrophage activity associated with RP is further supported by the strong correlation coefficient between macrophage count and lac/pyr for the right lung (r = 0.86, p < 0.01) and the left lung (r = 0.85, p < 0.01) (Fig. 4e and f). This hypothesis also seems to be supported by previous evidence associating increase in lac/pyr with inflammation in other disease (arthritis) models [33]. The strong correlation between lac/pyr and macrophage count suggests that the enhanced lac/pyr seen in the irradiated cohort is likely due to up-regulation of macrophage activity and the associated inflammation in RP. Due to the small sample size for each cohort (n = 3), the t-tests presented above were performed under the assumption of unequal variance. Nonetheless, with a sample size of n = 3 for each cohort, the statistical power of this work is quite limited. Increasing the sample size should result in more statistical power and better quantification of lac/pyr and macrophage count to further support this hypothesis.

This proof-of-concept work demonstrates that early metabolic changes (over days) can be mapped using hyperpolarized 13C-pyruvate MRSI in a rat model of RP involving conformal radiotherapy. Increases in lac/pyr are highly correlated with macrophage count in both the irradiated and unirradiated lungs suggesting that part of the enhanced lac/pyr may be caused by an increase in the number and activity of infiltrating macrophages in response to inflammation. Future studies with larger sample size could focus on the use of hyperpolarized 13C-pyruvate MRSI to measure the regional effects on Radiation Pneumonitis arising from varying conformal radiotherapy (dose, dose rate, fractionation scheme, etc.) and intervention during the course of the injury for the mitigation of RP.

Conflict of interest
None.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.radonc.2013.11.016.

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