

# A Novel Absolute Radiation Dosimetry Approach Measuring Reactive Oxygen Species

J. Rahimian, Ph.D., M. Hitchler, Ph.D.

Radiation Oncology Department, Kaiser Permanente Los Angeles Medical Center, Los Angeles, California 90027

## Introduction

We hypothesized that the hypersensitivity response and therapeutic gain observed in Stereotactic Ablative Radiotherapy (SABR) is mediated, at least in part, through the increased production of Reactive Oxygen Species (ROS). In this study we present an improved standard for the absolute absorbed dose to water based on ROS produced in water samples specifically  $\text{H}_2\text{O}_2$  molecule produced post irradiation to ionizing radiation.

## Aim

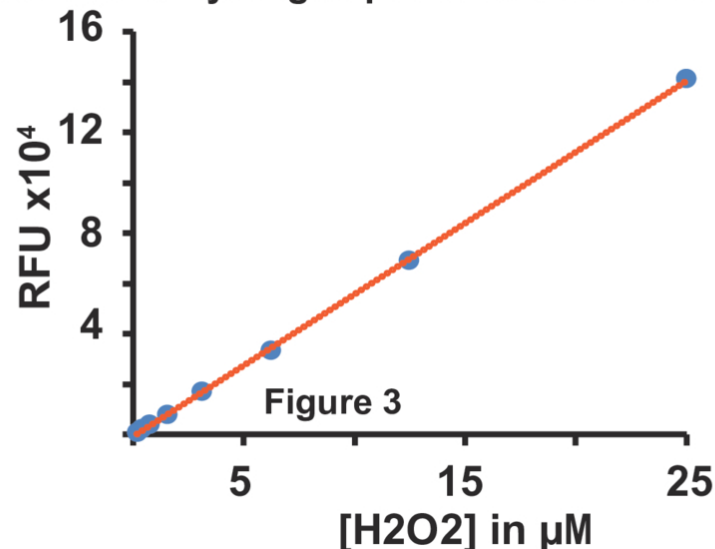
To develop a novel absolute radiation dosimetry approach using fluorometric technique to measure SABR's potential to generate  $\text{H}_2\text{O}_2$ .

## Methods

Hydrogen peroxide was quantified using the Resorufin  $\text{H}_2\text{O}_2$  Assay following the manufacturer's guidelines. To measure  $\text{H}_2\text{O}_2$  levels after exposure, 100  $\mu\text{l}$  of Ultra Trace water was exposed in a 10 x 10  $\text{cm}^2$  field, 100 cm SSD at  $d_{\text{max}}$  in solid water phantom. The dose linearity was measured exposing the samples to 10XFFF between 0.05 to 200Gy. Fluorescence was measured using a commercially available fluorometer. The absolute  $\text{H}_2\text{O}_2$  was calculated using a standard curve of known  $\text{H}_2\text{O}_2$  concentrations.

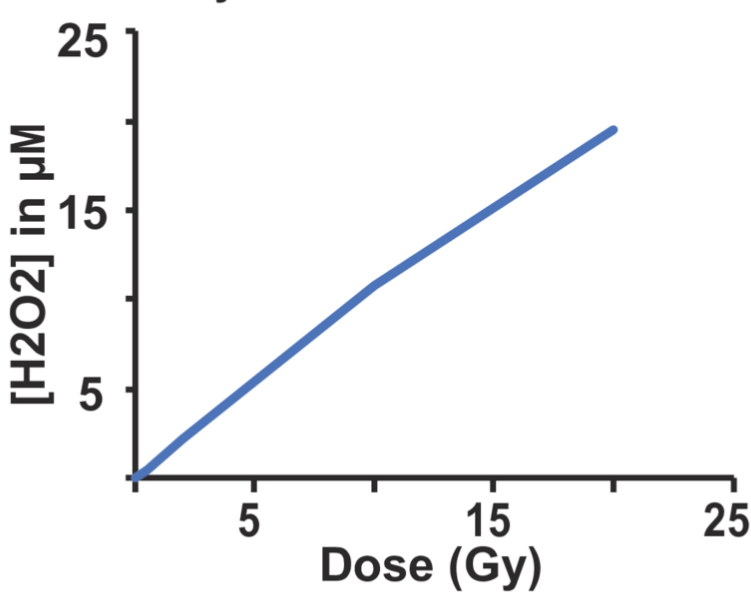
## Results

Figure 1: Resorufin fluorescence is linearly related to hydrogen peroxide concentration



A standard curve of hydrogen peroxide mediated resorufin was created using varying amounts of  $\text{H}_2\text{O}_2$  ranging from  $0.01\mu\text{M}$  –  $24.9\mu\text{M}$ . We observed a linear relationship between resorufin fluorescence over a broad range of  $\text{H}_2\text{O}_2$  concentrations (R<sup>2</sup> = 0.99).

Figure 2: Hydrogen peroxide production is directly related to absorbed dose



## Results

Figure 3



A linear relationship between Resorufin fluorescence and dose exists within a range 0.05 - 200Gy exposed to 10XFFF (Figure 2). Our measured absolute dose for various photon and electron energies was within +/-2% of the absolute dose. We determined the yield of  $\text{H}_2\text{O}_2$  created by radiation to be  $0.66\mu\text{M}/\text{Gy}$ . The results suggest this assay is sensitive and can detect exposures over a broad dose range. Figure 3 compares the vials exposed to various doses of 10XFFF and 6XFFF. The intensity of the color represents the  $\text{H}_2\text{O}_2$  concentration in the samples produced by radiation exposure that is quantified by a commercial fluorometer.

## Summary

- Hydrogen peroxide is formed during the irradiation of water
- Our fluorescence assay can detect a broad range of  $[\text{H}_2\text{O}_2]$ .
- Radiation produced  $\text{H}_2\text{O}_2$  at a constant yield over broad dose range.

## Conclusions

We developed a sensitive fluorometric technique to measure absolute dose to water by quantifying the  $\text{H}_2\text{O}_2$  production. These results show our technique is capable of linearly measuring dose for a broad range of photon and electron energies, dose and dose rates, suggesting this dosimetry method more accurate than conventional methods especially during SABR or Flash therapy. Further research is warranted.

## References

Zhou M, *et al* A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. *Anal Biochem.* 1997;253(2):162-168.

## Acknowledgements

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## Contact Information

Javad.X.Rahimian@kp.org