

Lipid hydroperoxides (LPO) extraction from tumor and non-tumor rats tissues Cancer Research Laboratory, University Hospital of Tours,

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CONTEXT

The fundamental and clinical project of our research unit is positioned at the junction of the two fields "cancer with a specialization in biochemistry and breast cancer. Our research unit has described the potential benefit of the clinical use of lipid nutrients in order to increase the efficiency of cancer treatment. Docosahexaenoic acid (DHA) has the potential to increase tumor sensitivity to chemotherapy with no sensitization of normal tissues. This study [1] was aimed at exploring the mechanism involved in this differential sensitization with a focus on oxidative stress, one of the main determinants involved in DHA enhancement of anthracycline-based chemotherapy.

MATERIAL

- Precellys 24 homogenizer with Cryolys cooling device to have a constant temperature of 4°C within the homogenization chamber using liquid nitrogen.
- Precellys lysing kit: 03961-1-009 (CKmix).
- Sample: ~100 mg of frozen tumors, intestine, liver, and heart from rats treated with DHA + epirubicin and from control rats (palm oil, no chemotherapy).
- Buffer: ice-cold distilled water.

PROTOCOL

- Precellys setting: 6500 rpm, 3x20sec, 50sec break.
- Lipid hydroperoxides (LPO) were extracted and assayed with a lipid hydroperoxides assay kit according to the manufacturer's instructions (Kit no. 705003, Cayman Chemical Company, Ann Arbor, MI, USA).

[1] Hajjaji N. et al., Tumor and non-tumor tissues differential oxidative stress response to supplemental DHA and chemotherapy in rats. CCP 2012. chemotherapy in rats. CC DOI10.1007/s00280-012-1884-0

RESULTS

Overall, our results showed that supplemental DHA during an anthracycline-based chemotherapy selectively increased tumor level of LPO. In fact, at baseline (control group) a similar level of LPO was in tumors, liver, heart, and intestine. Supplementing animals with DHA during chemotherapy increased the level of LPO in tumors while no change in LPO level was detected in liver, heart, or intestine (figure 1), even though their enrichment with DHA was larger than that of tumors. Enzyme activity assays showed a differential change in antioxidant defenses between tumors and other tissues. This differential handling of oxidative stress between tumors and other tissues might be a mechanism contributing to the absence of toxicity of DHA supplementation to normal tissues during chemotherapy.

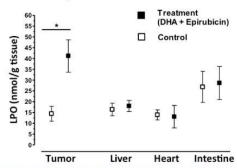


Figure 1: LPO level in tumor and non-tumor tissues at baseline (control rats) and in response to treatment with DHA and epirubicin.

During this study, the Cryolys guaranteed a constant cool temperature within the homogenization chamber.



CONCLUSION

The combo Precellys&Cryolys cooling option are suitable and reliable systems to homogenize a large range of rats tissues and tumors to investigate molecular and cellular mechanisms of action of lipids. In our rat model, an efficient and equably homogenization of tissues with the Precellys&Cryolys was a prerequisite for an optimal subsequent extraction and measure of lipid hydroperoxides in tissues.



For more details, please contact precellys@bertin.fr



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