The Toxicological Impact of Unconjugated Linoleic Acid

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Introduction

Linoleic acid, an omega-6 polyunsaturated fatty acid, is one of the most abundant fatty acids in the Western diet (Whelan, 2008). Linoleic acid, being a precursor for arachidonic acid, is associated with the production of pro-inflammatory eicosanoids such as prostaglandins, thromboxanes and leukotrienes (Johnson and Fritsch, 2012).

It is also suspected that linoleic acid may display potent pro-inflammatory activities, independent of its role as an arachidonic acid precursor. Various cell types have been shown to metabolise linoleic acid into other biologically active oxidation products such as leukotoxin (Fritch, 2008).

Project Aim

The primary aim of this investigation is to delineate the mechanism of action of dietary linoleic acid, specifically *cis*-9, *cis*-12, unconjugated linoleic acid (ULA), in relation to cytotoxicity and inflammation.

Methods and Materials

Using the epithelial cell line, HepG2, and the endothelial cell line, HUVEC, the cytotoxicity of varying concentrations of ULA (2mM to 10mM) have been assessed over a period of 24 and 48 hours using the colorimetric resazurin assay as an endpoint. Adenosine Triphosphate (ATP) was assessed in HepG2 cells treated with varying concentrations of ULA as an indicator of mitochondrial health using an ATP Assay Kit.

Results

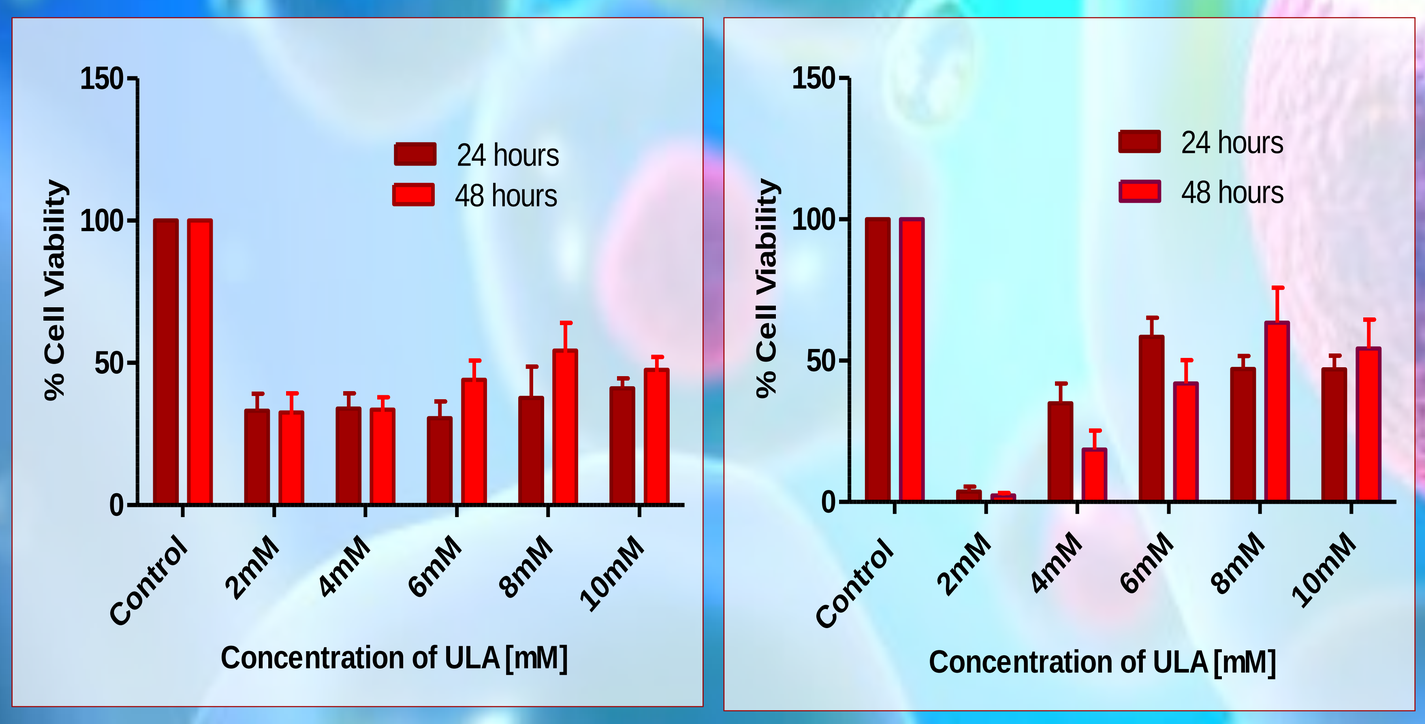

Figure 1. Percentage of viable HepG2 cells **(A)** and HUVEC cells **(B)** treated with varying concentrations of ULA over a period of 24 and 48 hours compared with the positive growth control (*n*=3).

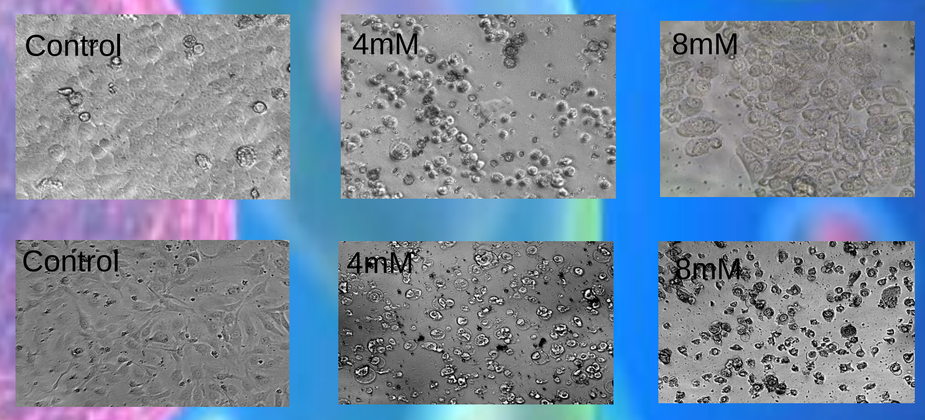

Figure 2. Effects of ULA treatment on the morphology of **(A)** HepG2 cells (x200) and **(B)** HUVEC cells (x100) at 24 hours. Control cells were grown in 0.5% BSA supplemented DMEM containing 1% IPA.

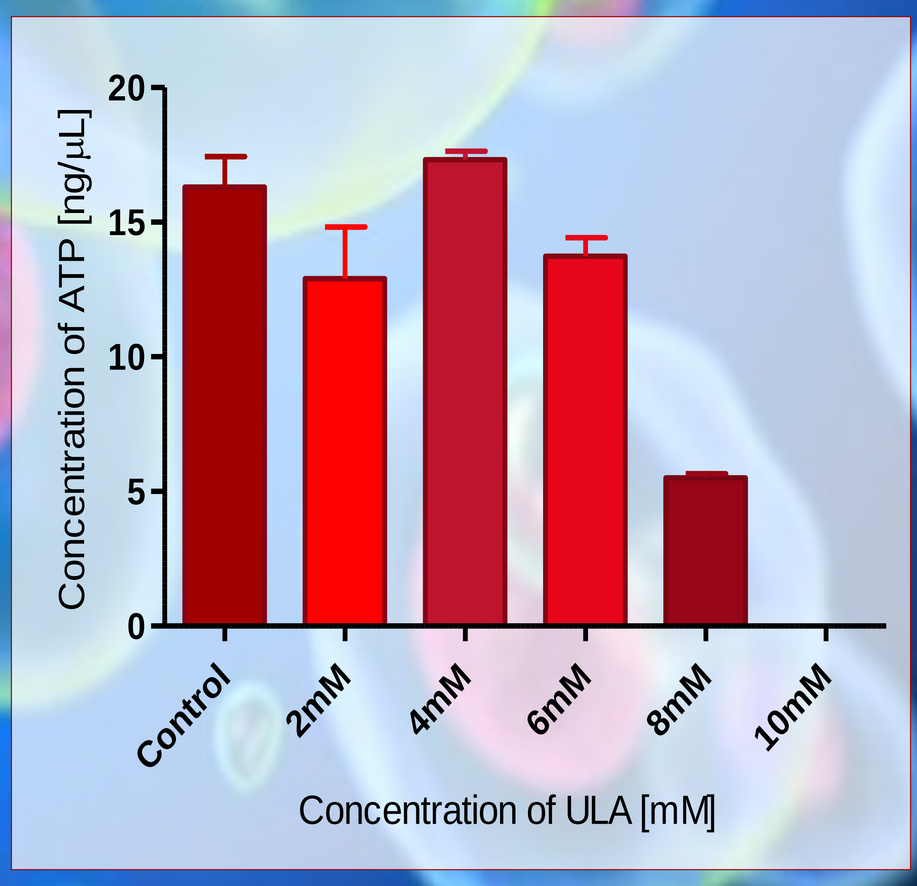

Figure 3. The concentration of ATP (ng/µL) produced by HepG2 cells treated with varying concentrations of ULA for a period of 21 hours (*n*=3).

Summary and Future Work

Initial cell viability assays indicated that elevated levels of ULA applied to both epithelial and endothelial cells had a significant inhibitory effect (*P=0.0001*) (Fig. 1) with a non-monotonic response being observed.

An Adenosine Triphosphate (ATP) assay was used to assess cellular and, more specifically, mitochondrial health. The data depicted in Fig. 3 denote that higher levels of ULA affected ATP production, a potential indicator of oxidative stress (Yamashina *et al,* 2009).

Future work will involve the use of ELISA to determine the impact of the analytes of interest on inflammatory biomarkers such as TNF-α and thromboxane A_2. Further analysis of the potential for ULA to induce the production of reactive oxygen species will be carried out through the assessment of superoxide dismutase activity within treated cells. Cellular fractionation will be carried out with the aim of identifying the cellular location of ULA within hepatic samples.

Connect & Discover

References

- Fritsch, K. L. (2008) Too much linoleic acid promotes inflammation – doesn't it? *Prostaglandins, Leukotrienes and Essential Fatty acids,* vol. 79, pp. 173 – 175.
- Johnson, G. H. and Fritsch, K. L., (2012) Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomised controlled trials, *Journal of the Academy of Nutrition and Dietetics,* vol. 112, pp. 1029 – 1041.
- Whelan, J. (2008) The health implications of changing linoleic acid intakes, *Prostaglandins, Leukotrienes and Essential Fatty acids,* vol. 79, pp. 165 – 167.
- Yamashina, S., Sato, N., Kon, K., Ikejima, K. and Watanabe, S., (2009) Role of mitochondria in liver pathophysiology, *Drug Discovery Today: Disease Mechanisms/Mitochondrial Mechanisms,* vol. 6, pp. 25 – 30.