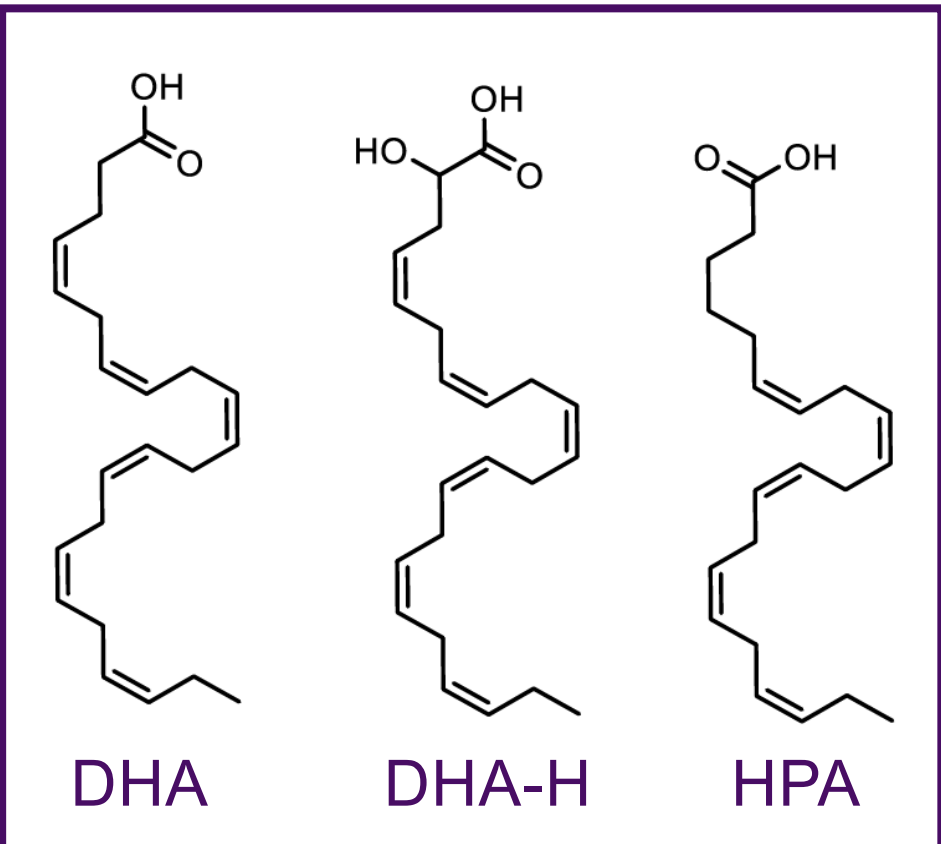


Astrocyte metabolism modulation by hydroxylated DHA leads to neuroprotection against excitotoxicity-induced neuronal cell death

Abstract #8712

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α -Hydroxy-Docosahexaenoic Acid (DHA-H) and Heneicosapentaenoic Acid (HPA), both ω -3 polyunsaturated fatty acids, show neuroprotection against Alzheimer’s disease (AD) in cellular and animal models. α -Oxidation of DHA-H into HPA is considered a key event in the neuroprotection exerted by DHA-H. DHA-H and HPA induce lactate production in cultured astrocytes and 5xFAD brain, leading to neuroprotection.



INTRODUCTION AND METHODS

RESULTS

CONCLUSIONS

Background: Available treatments for AD are limited in efficacy and based on decades-old molecular targets. Modulating brain metabolism has emerged as a promising new approach. Here, we examine the effects of the α -hydroxylated form of DHA (Docosahexaenoic acid), its metabolite HPA (Heneicosapentaenoic Acid), and the natural form of DHA as a control on astrocyte bioenergetics and neuroprotection.

Methods: DHA-H, HPA and DHA ω -3 fatty acids were provided by Laminar Pharmaceuticals (Spain). Lipid treatments were applied to mouse primary astrocytes or human U118 astrocytoma cells for 24 h. Lipid droplet formation and mitochondria colocalization were determined by confocal fluorescence microscopy in these cultures. Bioenergetic parameters, including OCR (Oxygen Consumption Rate) and ECAR (Extracellular Acidification Rate), were measured using the Seahorse XF96 (Agilent Technologies) in a kinetic assay over 90 minutes, generated by the sequential addition of oligomycin, FCCP, and rotenone/antimycin.

Neuroprotective effects were assayed in a neuronal model of excitotoxicity. HumanSH-SY5Y neuroblastoma cells were differentiated into a neuronal phenotype using retinoic acid and hBDNF. Neurons were treated with conditioned medium from U118 cells or exogenous L-lactate for 24 h, then subjected to NMDA/Ca²⁺ stimulation for 1 h to induce cell death by excitotoxicity.

Regarding animal treatments, 5xFAD mice received chronic oral treatment at a daily dose of 20 mg/kg (DHA-H or HPA) between 3 and 7 months of age (4 months of treatment). Lactate levels were measured in brain extracts of 5xFAD mice treated with the molecules and WT controls using a commercially available kit (Sigma) according to the manufacturer's instructions. Cognitive assessment of mice was addressed during the last month of treatment in a radial arm maze, where Working Memory Errors (WME), Reference Memory Errors (RME) and total errors (sum of WME and RME) were quantified.

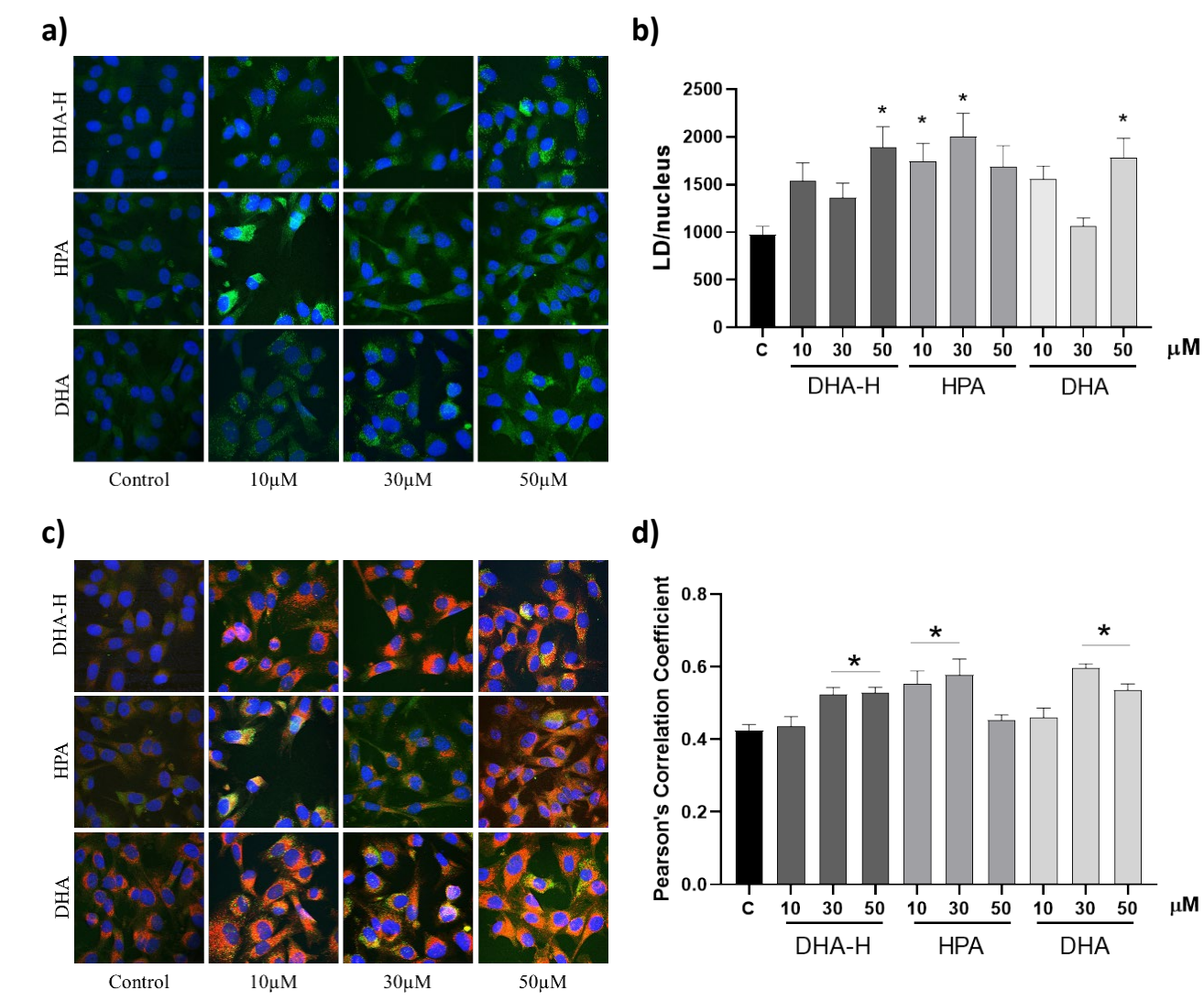


Figure 1. Lipid droplet accumulation and lipid droplet-mitochondria colocalization in U118 cells. (a) Representative confocal microscopy images of U118 cells showing lipid droplets (LDs) stained with BODIPY 493/503 (green) and nuclei counterstained with DAPI (blue). (b) Quantification of LDs (expressed as the number of LDs per nucleus) shows intracellular accumulation of LDs induced by the 3 treatments. (c) Representative confocal microscopy images showing LDs (BODIPY, green), mitochondria (TOM20, red), and nuclei (DAPI, blue), with LD-mitochondria colocalization visible as orange puncta. (d) LD-mitochondria colocalization using Pearson's correlation coefficient showed strong colocalization of formed LDs with mitochondria in the 3 treatments. Bars represent mean $\pm$ SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 compared to control (C) group.

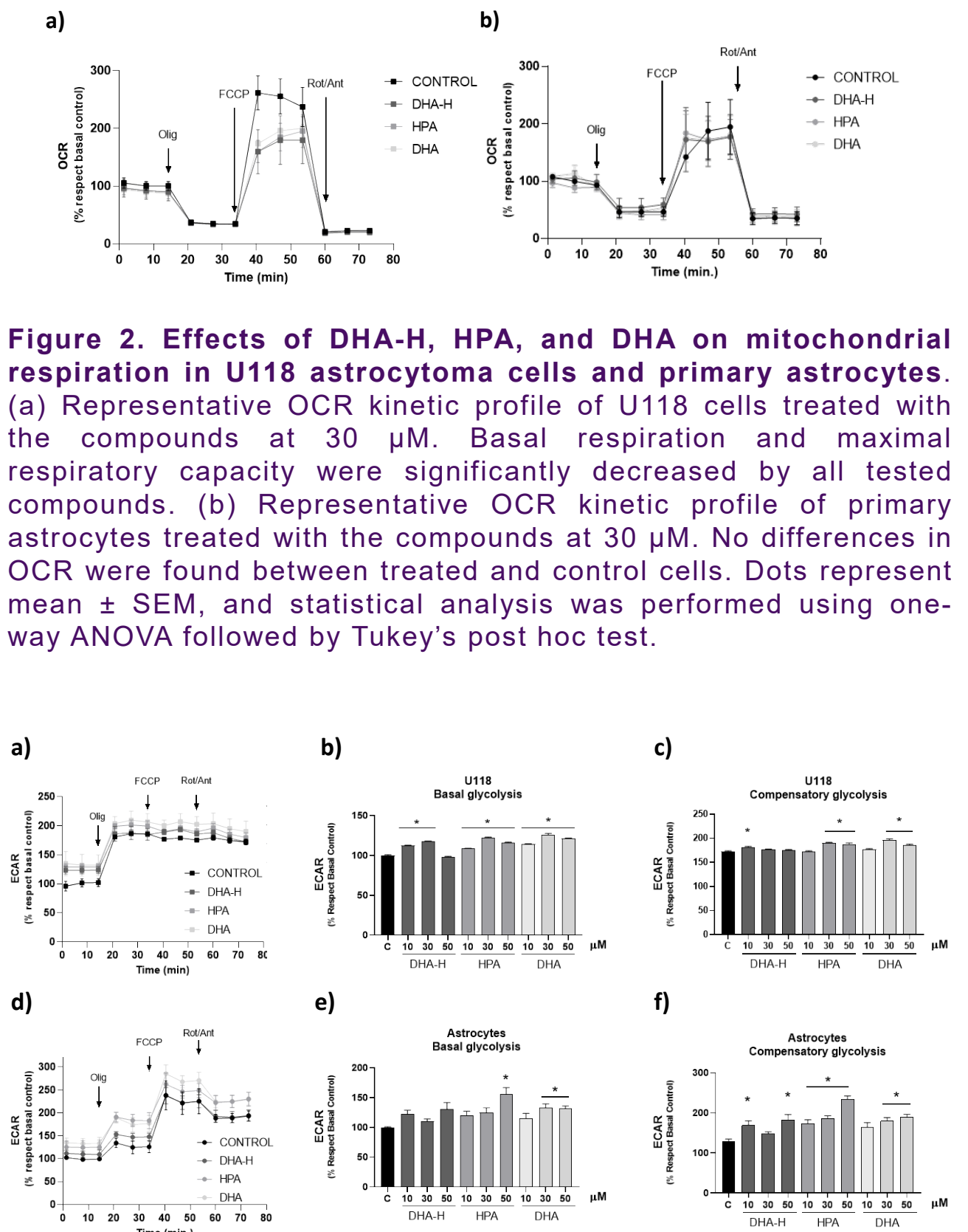


Figure 3. DHA-H, HPA, and DHA promote Glycolysis and lactate production in U118 astrocytoma cells and primary astrocytes. The extracellular acidification rate (ECAR), primarily associated with lactate production, of U118 cells and primary astrocytes was assessed after incubation with DHA-H, HPA, or DHA. Representative ECAR kinetic profile of U118 cells (a) or primary astrocytes (d) treated with the compounds at 30 μ M. Basal glycolysis and compensatory glycolysis were quantified in U118 cells (b and c) or primary astrocytes (e and f) for all experimental conditions. Bars represent mean $\pm$ SEM, and statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 compared to control (C).

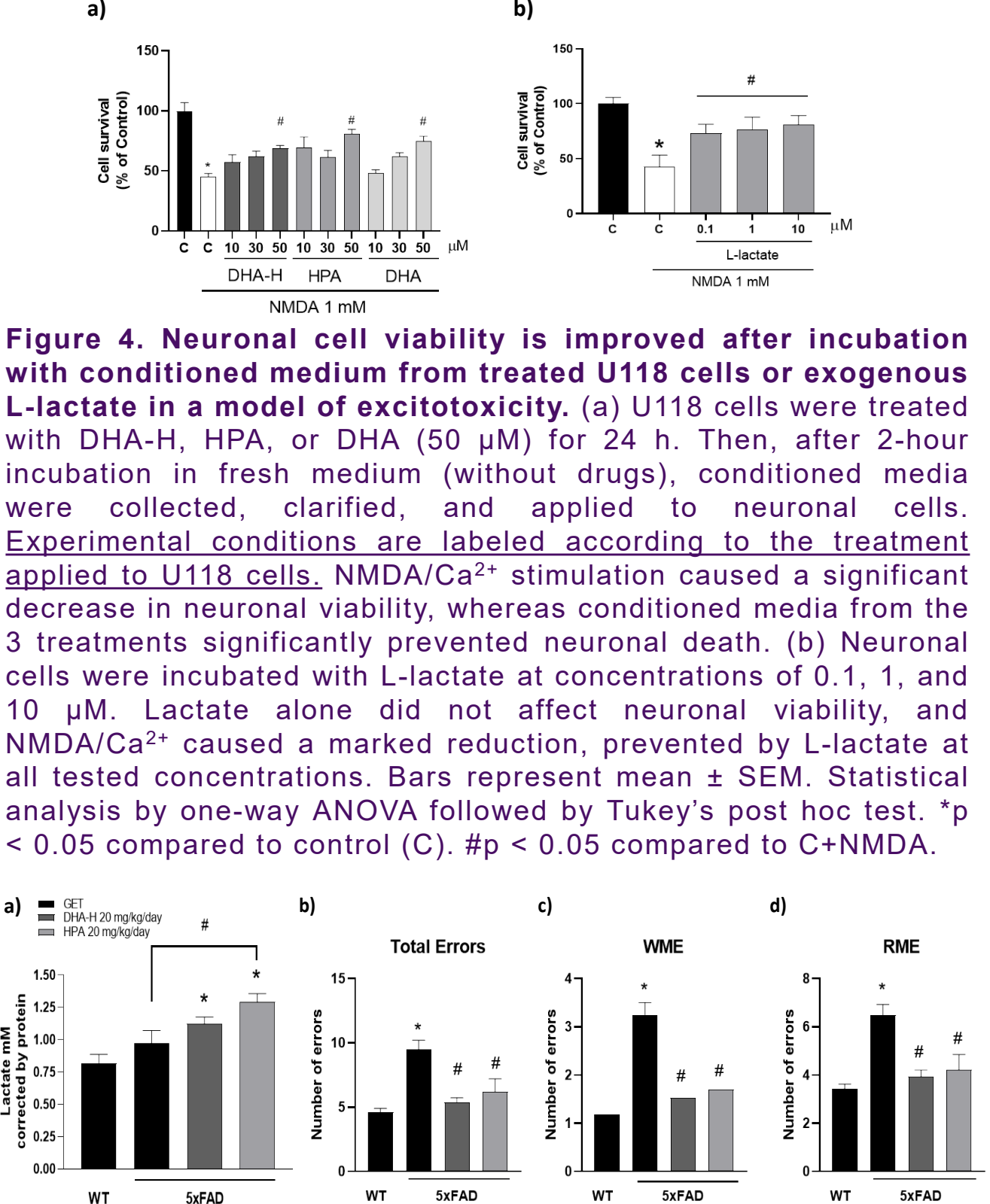


Figure 4. Neuronal cell viability is improved after incubation with conditioned medium from treated U118 cells or exogenous L-lactate in a model of excitotoxicity. (a) U118 cells were treated with DHA-H, HPA, or DHA (50 μ M) for 24 h. Then, after 2-hour incubation in fresh medium (without drugs), conditioned media were collected, clarified, and applied to neuronal cells. Experimental conditions are labeled according to the treatment applied to U118 cells. NMDA/Ca²⁺ stimulation caused a significant decrease in neuronal viability, whereas conditioned media from the 3 treatments significantly prevented neuronal death. (b) Neuronal cells were incubated with L-lactate at concentrations of 0.1, 1, and 10 μ M. Lactate alone did not affect neuronal viability, and NMDA/Ca²⁺ caused a marked reduction, prevented by L-lactate at all tested concentrations. Bars represent mean $\pm$ SEM. Statistical analysis by one-way ANOVA followed by Tukey's post hoc test. *p < 0.05 compared to control (C). #p < 0.05 compared to C+NMDA.

1. The omega-3 fatty acids studied in this work (DHA-H, Hpa and DHA) induced lipid droplet accumulation in astrocytoma cells. Formed lipid droplets strongly co-localized with mitochondria.
2. All the 3 molecules led to increased lactate levels in astrocytes. Mitochondrial respiration was not affected in primary astrocytes but significantly decreased in astrocytoma cells.
3. Drug-free, lactate-enriched medium from astrocytoma cells mimicked neuroprotection exerted by exogenous L-lactate in a neuronal model of excitotoxicity.
4. 5xFAD mice chronically treated with DHA-H or HPA accumulated L-lactate in the brain. The same mice displayed cognitive impairment, which can be prevented by chronic treatment with DHA-H or HPA.

FUNDING AND CONTACT

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