Electronic Supplementary Material (ESI) for Food & Function.

Supporting Information

List of Supporting information

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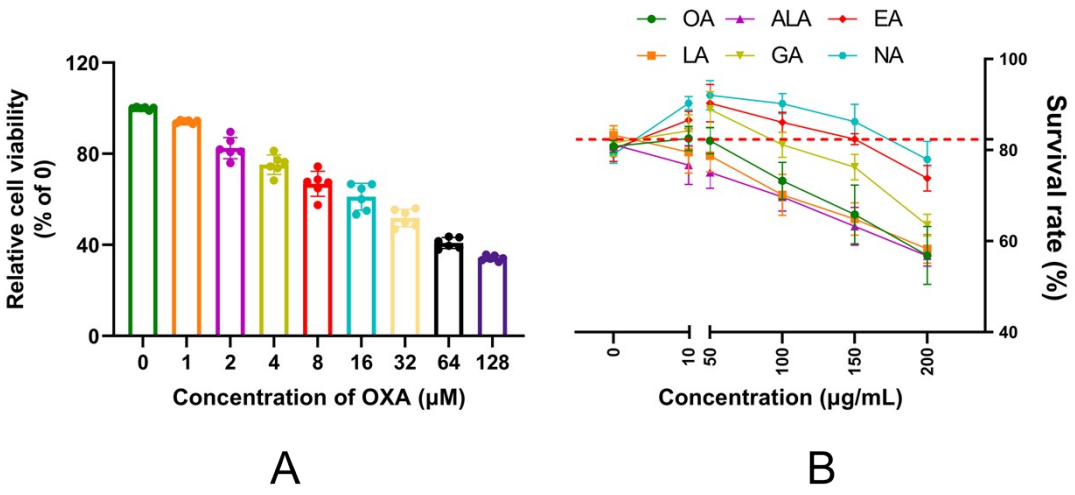

Effect of oxaliplatin on the viability of RSC96 cell (A) and fatty acids on the survival rate of zebrafish (B)

Determination of Fatty Acid Composition. To obtain the total fatty acid composition of ATSO, oil samples (60 mg) were vortexed after the addition of 3 mL of hexane and 1 mL of 2 mol/L KOH-CH_3OH solution. Subsequently, the reaction product was centrifuged for 10 s. Then, the upper phase was collected, and finally analyzed using gas chromatography analysis.

Animals' maintenance and treatment. Adult zebrafish are routinely maintained according to the standard methods of the CZRC. Zebrafish embryos were collected from spawning adult fish and washed three times with E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$). Subsequently, after zebrafish were cultured in the above medium for 72h, 3dpf zebrafish larvae were collected for further experiments.

The methods for modeling demyelination in zebrafish, as well as the treatments involving ATSO and various fatty acids are outlined below. Initially, 3dpf zebrafish were subjected to a four-day with varying concentrations of FOLFOX (0.125X, 0.25X, 0.5X,1X,2X,4X FOLFOX) in E3

medium. (The composition of 1X FOLFOX is as follows: 4.2mM 5-Fluorouracil, 0.18 mM folinic acid, 0.08 mM oxaliplatin). Zebrafish were then washed three times with fresh E3 medium to minimize any lingering effects of the modeling drugs and transferred to fresh E3 medium until 7dpf for further analysis. During this period, daily records were maintained regarding the mortality of zebrafish in each experimental group. Based on these initial experiments, suitable concentrations of FOLFOX were selected for subsequent experiments involving ATSO and fatty acids intervention. In the ATSO and fatty acids intervention experiment, different concentrations of ATSO and fatty acids (10 μg/mL, 50 μg/mL, 100 μg/mL, 150 μg/mL and 200 μg/mL in 1% DMSO) were employed. A minimum of 60 larvae per group is required. After 24 hours of continuous incubation, the larvae were utilized for analysis.

Tail fin axon analysis. Larvae were anesthetized with 0.03% tricaine and fixed with 2% (w/v) paraformaldehyde- PBS for 4 h at room temperature. After fixation, larvae were washed repeated three times with PBS/0.05% Tween 20/1% DMSO/1% Triton-X-100 (PBSTDT). Subsequently, larvae were dehydrated on a gradient with pre-cooled 25%, 50%, 75%, and 100% methanol-water solutions and incubated overnight at -20℃. The next day, the pre-cooled acetone solution was replaced and incubated for 10 min at -20℃. The fish were then washed twice with ddH_2O for 1 min each. Then, larvae were washed for 10 min in TBS/0.05% Tween/1% DMSO/1% Triton X-100 (TBSTDT). Larvae was incubated with 10% (v/v) goat serum/TBSTDT for 1 h at room temperature, then incubated with anti-acetylated microtubule protein antibody dilution (1:1500) at 4°C. After incubation for about 14 h, the samples were washed 10 times repeatedly with PBSTDT for 30 min. After washing, the samples were incubated with Alexa 488 goat-anti-mouse antibody dilution (1:1000) overnight at 4℃. Subsequently, zebrafish were washed 3-5 times

with TBS/ 0.05% Tween for 15 min each time. Subsequently, zebrafish were mounted in a sealed chamber containing 1.2% low melting point agarose and imaged using laser confocal microscopy. Z-stack imaging of larvae was performed using a 40X objective lens and pictures were taken every 2 μm. Maximum stacks of the taken images were projected to a single image using Photoshop software, and regional axon densities were quantified using the semi-automatic filament function in imaris7.4.2 software.

Preparation of intervention drugs. Fatty acids or ATSO (molar mass: 290.8, molar mass was calculated as the average molar mass of ATSO) were mixed with 0.03g sodium hydroxide in 10 mL PBS solution at a molar ratio of 1:1.5 and heated with water bath for 1-2 h at 90℃ to obtain a single or mixed sodium fatty acid-PBS solution. After cooling to room temperature, 4 mL above single or mixed sodium fatty acid-PBS solution was taken and mixed with 6 mL 10% (w/w) BSA-PBS to obtain 20 mM single or mixed sodium salt of fatty acids intervention. The samples were filtered through a water-based membrane and diluted with treatment medium consisting of DMEM, 100 units/mL penicillin, and 100 μg/mL streptomycin to the intervening concentration.

Cell culture and treatment. The RSC96 cell line was purchased from National Collection of Authenticated Cell Cultures (NCACC). RSC96 cells were cultured in complete cell culture medium consisting of DMEM with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin and cultured in a 37℃ and 5% CO_2 humidified incubator. The cell culture medium was replaced every day, and confluent cells were passed every 2 days with trypsinization.

To evaluated cytotoxicity of SSFAs and OXA, cells were seeded at a density of 3×10^4 cells per well in 96-well plates. When cells were about 80% confluent, RSC96 cells were treated with treatment medium with 0, 50, 100 and 200 μM different SAFAs or 0, 1, 2, 4, 8, 16, 32, 64 and 128

μM OXA.

To evaluate the effect of SSFAs on OXA-induced RSC96 cell proliferation, RSC96 cells were seeded at 3×10^4 cells/well on 96-well cell culture plates overnight. The cells were exposed with 32 μM oxaliplatin for 24 h and then treated with SAFAs for another 24 h. In addition, RSC96 cells were cultured in treatment medium as a control group.