VERY LONG CHAIN FATTY ACID-INDUCED CELL DEATH WITH AUTOPHAGIC CHARACTERISTICS ON OLIGODENDROCYTES

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Introduction: Inherited peroxisomal disorders mostly develop a neuronal phenotype, suggesting important functions of this organelle in nervous tissue. Thus, patients with peroxisomal dysfunctions can present severe neurological abnormalities which can lead to brain demyelination in certain forms of peroxisomal leukodystrophies such as X-linked adrenoleukodystrophy (X-ALD) and pseudo-neonatal adrenoleukodystrophy (P- NALD) associated with ABCD1 and ACOX1 deficiency, respectively. Consequently, in X-ALD and P-NALD patients, increased plasmatic and tissue levels of C24:0 and C26:0 are found. It was therefore of interest to characterize the type of cell death induced by VLCFA on murine oligodendrocyte cells which are myelin forming cells in the central nervous system.

Methods: Murine oligodendrocyte cells (158N) were treated for 24 h or 48 h with VLCFA (5; 10 and/or 20 µM) seeded at 120 000 cells/mL. The impact of VLCFA on cell viability was evaluated by flow cytometry with $DiOC_6(3)$ (Δψm measurement) and acridine orange (lysosomal integrity), quantification of LDH activity released in the culture medium by damaged cells and/or identification of dead cells with propidium iodide. In addition, to characterize cell death (apoptosis versus necrosis), analysis of nuclear morphology was performed after staining with Hoechst 33342 (normal cell are characterized by round and regular nuclei, necrotic cells by nuclei with irregular sizes and shapes, apoptotic cells by nuclei with perinuclear chromatin, condensed chromatin and/or fragmented nuclei). To characterize autophagy, the presence of LC3-II (autophagic marker) was analyzed by Western blotting.

Results: In those conditions, on 158N cells, a decrease of cell viability was observed with C24:0 or C26:0 used at 10 µM, or C24:0 (5 µM) + C26:0 (5 µM)) (**Figure 1A**). These VLCFA concentrations are in the range of those found in the plasma of X-ALD. These VLCFA trigger an induction of cell death (**Figure 1B**) which was characterized by a decrease of Δψm and an altered lysosomal integrity (**Figure 2 A/B**). In addition, on mitochondria, the impact of VLCFA was studied by analyzing the expression of mitochondrial complex subunits : no marked effect was observed (**Figure 3**). No apoptotic cells were found with C24:0 and C26:0 (Absence of cells with condensed and/or fragmented nuclei (**Figure 4**). Moreover, on 158N cells, some characteristics of autophagy were observed, especially the presence of LC3-II detected with C24:0 and C26:0 at 20 µM and with C24:0 (5 µM) + C26:0 (5 µM) (**Figure 5**).

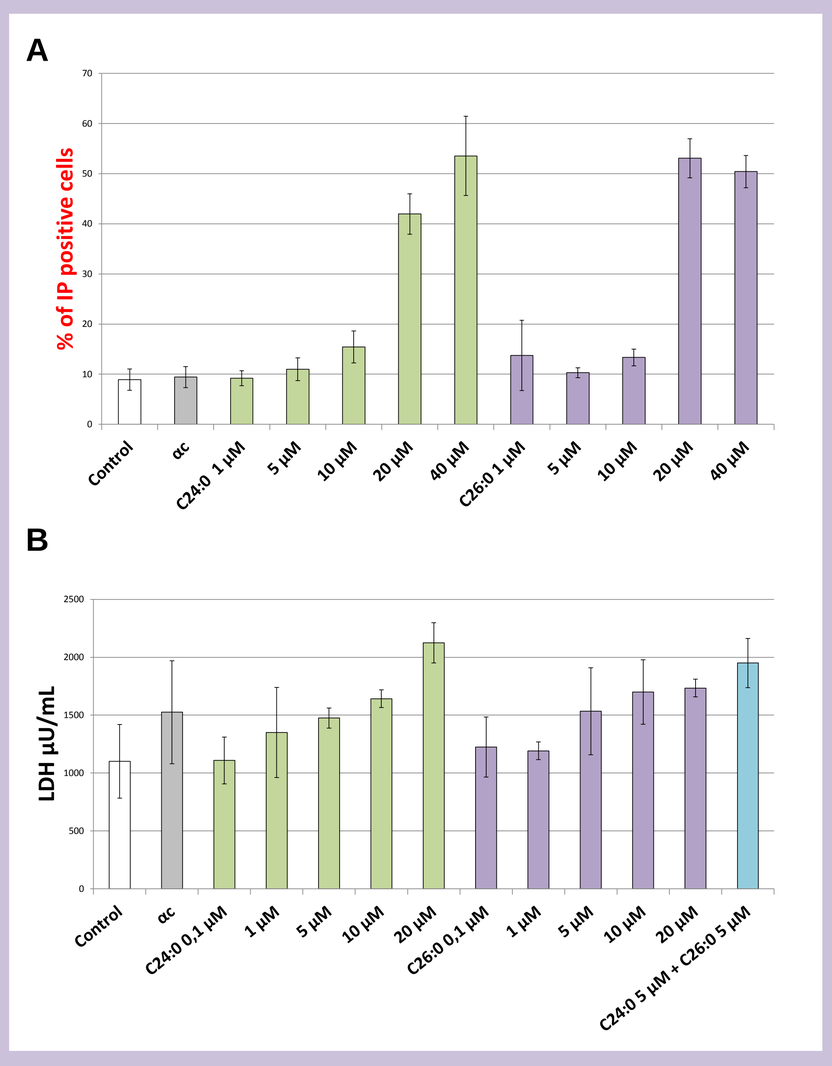


Figure 1 : Evaluation of cell viability on 158N murine oligodendrocytes treated with C24:0 and C26:0 : identification of dead cells with propidium iodide (PI) (A) and quantification of LDH activity (B). αc : α-cyclodextrin

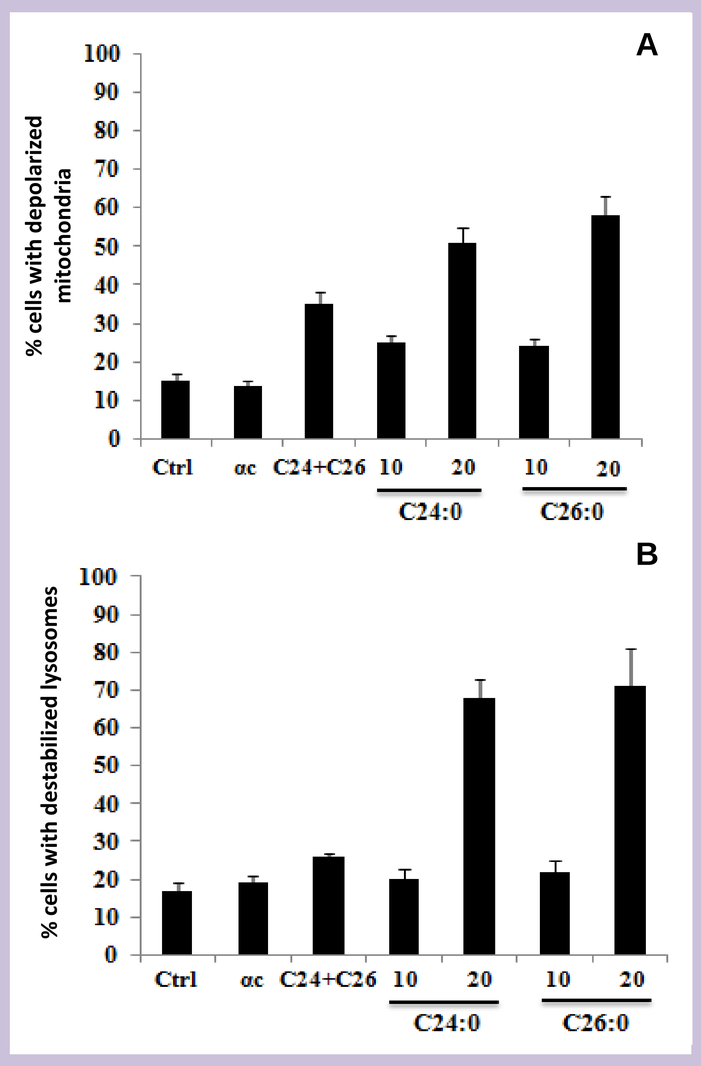
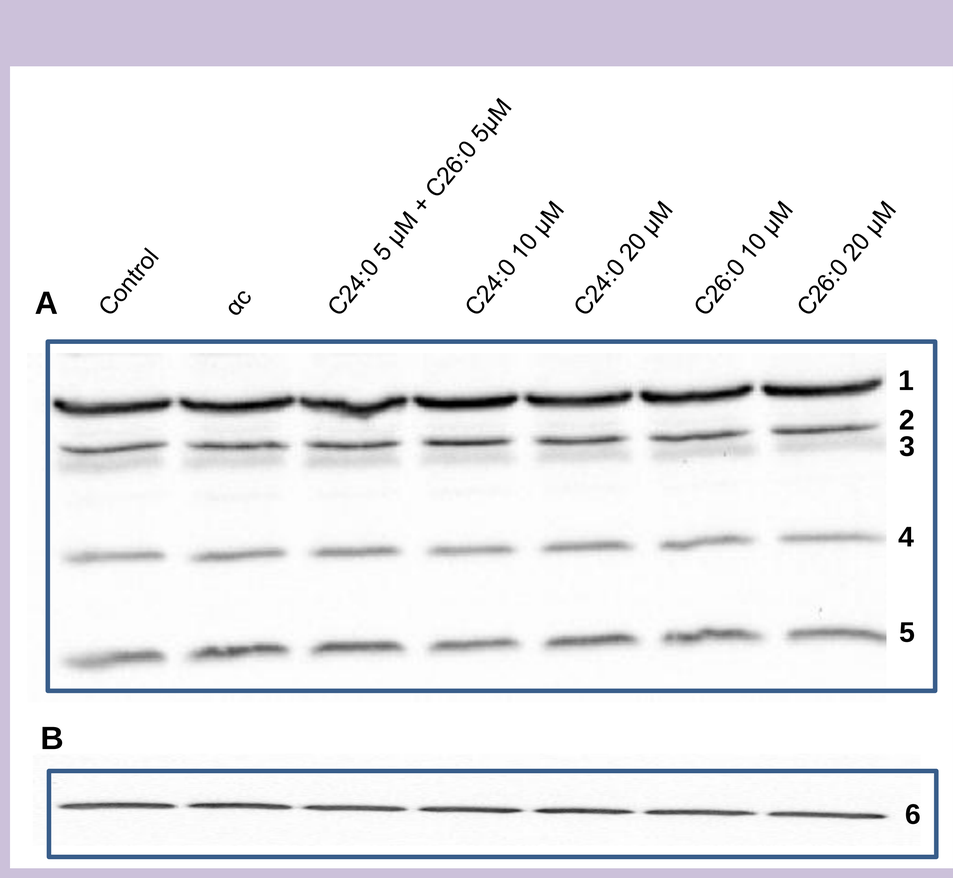


Figure 2 : Characterization of cell death induced by VLCFA on 158N murine oligodendrocytes treated with C24:0 and C26:0 at 10 and 20 µM : evaluation of Δψm (A) and lysosomal integrity (B).



1 Complex V (ATP synthase subunit alpha (53 kDa))
2 Complex III subunit core 2 (47 kDa)
3 Complex II subunit (30 kDa)
4 Complex IV subunit II (24 kDa)
5 Complex I subunit NDUFB8 (20 kDa)
6 Actin

Figure 3 : Analysis of the effects of C24:0, and C26:0 on the expression of mitochondrial complex subunits. Western blot of 158N cells cultured for 48 h in the absence (control) or presence of α-cyclodextrin, or VLCFA (C24:0, or C26:0; 5,10 and 20 µM). Specific antibodies (MitoProfile Total OXPHOS Murine WB Antibody Cocktail, Abcam/Mitosciences) (A) and anti-actin (Sigma) (B) were used.

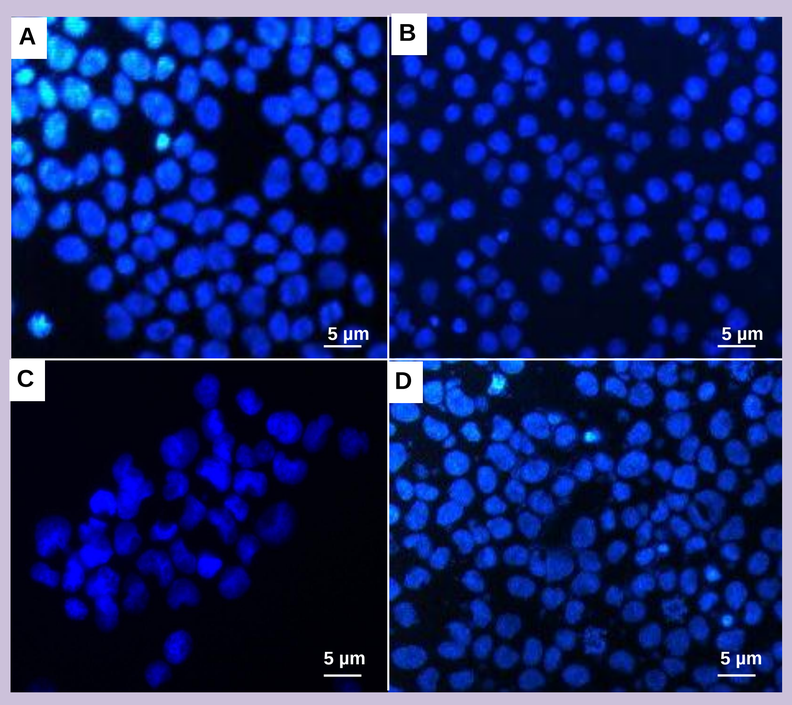


Figure 4 : Characterization of cell death apoptosis/necrosis after staining with Hoechst 33342 on 158N murine oligodendrocytes : after 24h of treatment **A**: 158N control cells, **B:** α-cyclodextrin (1 mM), **C:** C24:0 20 µM, **D:** C26:0 20 µM.

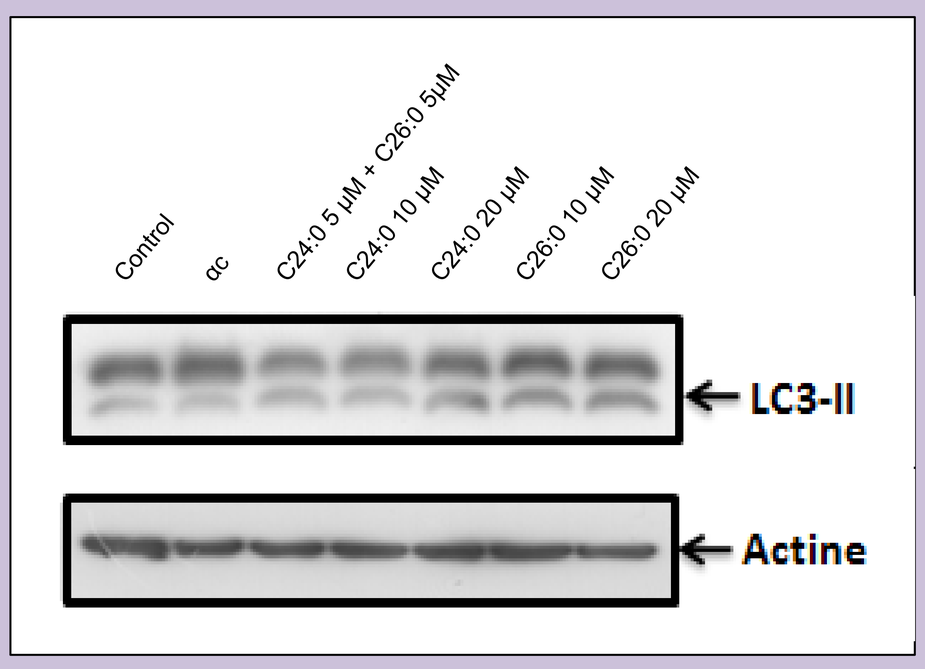


Figure 5 : Characterization of autophagy by western blotting on 158N murine oligodendrocytes. Western blot of 158N cells cultured at 24h in the absence (control cells) or presence of α-cyclodextrin (1 mM), C24:0 (10 and 20 µM), or C26:0 (10 and 20 µM) and a mixture of C24:0 and C26:0 at 5 µM.

Conclusion

✓ VLCFA (C24:0 and C26:0) are able to trigger cell death on 158N cells associated with autophagic characteristics

✓ It remains to define whether autophagy can be amplified by ABCD1 deficiency, and whether autophagy contributes to cell death or constitutes a defense mechanism.