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Incidence of Abcd1 level on the induction of cell death and organelle dysfunctions triggered by very long chain fatty acids and TNF-α on oligodendrocytes and astrocytes

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ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is characterized by ABCD1 deficiency. This disease is associated with elevated concentrations of very long chain fatty acids (C24:0 and C26:0) in the plasma and tissues of patients. Under its severe form, brain demyelination and inflammation are observed. Therefore, we determined the effects of C24:0 and C26:0 on glial cells:oligodendrocytes, which synthesize myelin, and astrocytes, which participate in immune response. So, 158N murine oligodendrocytes, rat C6 glioma cells, rat primary cultures of neuronal–glial cells, and of oligodendrocytes were treated for various periods of time in the absence or presence of C24:0 and C26:0 used at plasmatic concentrations found in X-ALD patients (1–5 μM) and higher (10, 20, 40 μM). To evaluate the importance of extrinsic and intrinsic factors, the part taken by TNF-α and reduced Abcd1 level was studied. Whatever the cells considered, no effects on cell growth and/or viability were detected at 1–5 μM, more or less pronounced effects were identified at 10 μM, and an induction of cell death with increased permeability to propidium iodide and loss of transmembrane mitochondrial potential was observed at 20–40 μM. On 158N, cell death was characterized by (i) an increased superoxide anion production at the mitochondrial level; (ii) the presence of vacuoles of different sizes and shapes; a destabilization of lysosomal membrane and a cytoplasmic redistribution of lysosomes; (iii) a modulation of Abcd3/PMP70 and Acox-1 protein expression, and a decrease in catalase activity at the peroxisomal level. When TNF-α was combined with C24:0 or C26:0 and used on 158N cells, C6 cells, and on 158N cells after siRNA mediated knockdown of Abcd1, no or slight potentiation was revealed. Thus, on the different cell models used, an induction of cell death with marked cellular dysfunctions at the mitochondrial, lysosomal, and peroxisomal levels were found with C24:0 and C26:0 at 20 μM and higher. However, in our experimental conditions, plasmatic concentrations of these fatty acids were unable to induce cell death, and organelle dysfunctions on oligodendrocytes and astrocytes, and additional intrinsic and environmental factors, such as reduced Abcd1 level and/or TNF-α, were ineffective to potentiate their side effects.

1. Introduction

With an incidence of around 1/20,000 males, X-linked adrenoleukodystrophy (X-ALD, OMIM 300100) is the most common monogenic leukodystrophy and peroxisomal disorder (Berger and Gärtner, 2006). This neurometabolic demyelinating disease is associated with a gene defect mapping to Xq28, leading to the functional loss of the peroxisomal ATP-binding cassette transporter type D (ABCD1), also named adrenoleukodystrophy protein (ALDP) (Mosser et al., 1993; Dubois-Dalcq et al., 1999). To date, more than 500 different mutations have been reported in the *ABCD1* gene (Kemp et al., 2001; see also X-ALD database: www.x-ald.nl). In adult mouse and human brain, ABCD1 expression has been described in astrocytes, microglial cells, and oligodendrocytes (Fouquet et al., 1997), and its presence in neurons appears to be limited to certain subtypes (Fouquet et al., 1997; Höftberger

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et al., 2007). ABCD1 has been proposed to play a crucial role in the transport of very long chain fatty acids (VLCFAs), or their coenzyme A derivatives, to peroxisomes where they are degraded by β-oxidation (Baes and Aubourg, 2009). This is based on the plasmatic and tissular accumulation, most notably in the brain and adrenal cortex (Wanders and Waterham, 2006), of saturated and monounsaturated VLCFA (tetracosanoic acid (C24:0), hexacosanoic acid (C26:0) (C26:1)) (Valianpour et al., 2003; Kemp and Wanders, 2007). Clinically, X-ALD is a complex inherited disease where the same mutation in the X-ALD gene (*ABCD1*) can lead to very distinct phenotypes, a fatal childhood cerebral ALD (CCALD) or the less severe adult disease of adrenomyeloneuropathy (AMN) (Kemp and Wanders, 2007). CCALD is characterized by multifocal inflammatory demyelination of the central nervous system (CNS), adrenal insufficiency, and a rapid fatal outcome (Ferrer et al., 2010). AMN affects adults and is characterized by a pure myelopathy and peripheral neuropathy. About 35% of AMN patients subsequently develop cerebral demyelination, and share the same poor prognosis as children with cerebral ALD. So far, no phenotype–genotype correlations have been established, suggesting that modifier genes or environmental/epigenetic/stochastic factors modulate the clinical outcome of the disease. Given the wide phenotypic variation in humans and that mice with the ALD mutation do not develop neurologic symptoms or demyelination at 1 year of age despite the presence of VLCFA (C24:0 and C26:0) in the brain (Kobayashi et al., 1997), it can be hypothesized that VLCFA accumulation is not sufficient for the pathogenesis of X-ALD (Hudspeth and Raymond, 2007). So, although the genetic basis of X-ALD has been investigated in detail (Kemp et al., 2001), the impact of VLCFA on neural cells is still not well known. Indeed, whereas the cytotoxic effects of middle and long chain fatty acids have been quite thoroughly studied, only few investigations are available on the side effects of VLCFA (Singh and Pujol, 2010; Wanders et al., 2010). It has been suggested that the incorporation of VLCFA by neuronal cells, and the presence of these fatty acids in their environment, might lead to dysfunctions and cell death. Indeed, in X-ALD, the identification of dead cells in brain lesions (Feigenbaum et al., 2000; Eichler et al., 2008) supports the belief that the death of oligodendrocytes, which are myelin-synthesizing cells (Baumann and Pham-Dinh, 2001), and of microglial cells (Eichler et al., 2008), which are known to produce TNF-α in stress conditions, might contribute to the demyelination process observed in severe forms of X-ALD (Ferrer et al., 2010). In addition to cell death of oligodendrocytes and glial cells, neuroinflammation associated with cytokine secretion (including interferon-γ, IL-1α, IL-2, IL-6, granulocyte macrophage – colony stimulating factor (GM-CSF), and TNF-α) might also constitute a major event contributing to trigger brain lesions in X-ALD patients (Paintlia et al., 2003; Ferrer et al., 2010; Singh and Pujol, 2010). Among these cytokines, TNF-α has some interest. Indeed, it has been reported to favor VLCFA accumulation in rat C6 glioma cells (Khan et al., 1998), and it is also known to favor apoptosis on various cell types, including neural cells (Lorz and Mehmet, 2009), via death receptors (Aktas et al., 2007). Currently, as mitochondria are known to play active roles in the mode of cell death triggered by biological, physical, and chemical agents (Kroemer et al., 2007), the influence of VLCFA on mitochondrial functions has already been studied. At the nonphysiological plasmatic concentrations studied (10, 20, and 40 μM), cell death observed on mixed primary cultures of rat astrocytes, oligodendrocytes, and neurons isolated from the hippocampus was detected mainly with C24:0 and C26:0 used at 20 and 40 μM. At these concentrations, a loss of transmembrane mitochondrial potential ($\Delta\Psi_m$) was found when measured with Rhodamine 123 (Hein et al., 2008). Similarly, mitochondrial alterations have been reported in a mouse model for Zellweger syndrome (Pex5 knockout mice) (Baumgart et al., 2001), whereas other results obtained from various cell types of mice lacking Abcd1 show neither impaired mitochondrial β-oxidation nor reduced activity of the respiratory chain (Oezen et al., 2005).

Another important organelle, playing important roles in cell death, is the lysosome (Yamashima and Oikawa, 2009). In comparison to untreated cells, we observed, using transmission electron microscopy, an increased number of lysosomes of various sizes and shapes on C24:0- and C26:0-treated 158N murine oligodendrocytes when these fatty acids were used at 20 μM (Kahn et al., 2011). Thus, it is tempting to speculate that the lysosome, involved in various types of cell death activated by different agents (Boya and Kroemer, 2008), might constitute a potential target of VLCFA.

Although there is currently some evidence that the lack of peroxisomes in oligodendrocytes and astrocytes has dramatic consequences on myelinization and inflammation in the CNS (Kassmann et al., 2007; Bottelbergs et al., 2010), no data are available on the effects of X-ALD-associated VLCFA on peroxisomal biogenesis, on enzymes involved in peroxisomal β-oxidation (especially Acox-1, which is the rate-limiting enzyme of peroxisomal β-oxidation (Oaxaca-Castillo et al., 2007; Vluggens et al., 2010)), and on peroxisomal functions (particularly on catalase activity, a peroxisomal enzyme that is one of the most important intracellular enzymes in the detoxification of the oxidant hydrogen peroxide and of some xenobiotics (Kirkman and Gaetani, 2007)).

Therefore, as VLCFA are increased in plasma and tissues, including the brain, of X-ALD patients, and as the inflammatory environment may contribute to the development of brain lesions in these patients, the aim of the present study was to characterize the impact of VLCFA (C24:0, C26:0) associated or not with TNF-α, on cell viability and/or on the organelles (mitochondria, lysosomes, and peroxisomes) of oligodendrocytes and astrocytes. To this end, based on C24:0 and C26:0 concentrations, that we measured in the plasma of X-ALD patients with different forms of the disease, and on those used in previous investigations (Fourcade et al., 2008, 2010; Hein et al., 2008), wild type 158N murine oligodendrocytes, which are well-differentiated murine oligodendrocytes (Baarine et al., 2009), 158N cells with reduced Abcd1 level, rat C6 glial cells (frequently employed to study X-ALD physiopathology (Khan et al., 1998; Gondcaille et al., 2005)), rat primary cultures of neuronal–glial cells, and rat primary cultures of oligodendrocytes were used and cultured for 24, 48, and/or 72 h in the absence or presence of C24:0 and C26:0 without or with TNF-α used at a concentration that we measured in the plasma of CCALD patients.

2. Methods

2.1. Cell cultures of murine oligodendrocytes (158N), rat C6 glioma cells, and treatments

Murine oligodendrocytes (158N) were immortalized with the SV40 large T-antigen derived from Tabby male (Ta/Y) control mice (Feutz et al., 2001). These cells have certain characteristics of differentiated oligodendrocytes (Baarine et al., 2009). Cells were seeded at 5000–10,000 cells/cm^2 either in 75-cm^2 culture flasks or in Petri dishes (100 mm in diameter), cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% antibiotics. Rat C6 glioma cells were seeded at 250,000 cells/mL either in 75-cm^2 culture flasks or in Petri dishes (100 mm in diameter). They were grown to confluence in DMEM/F12 containing 10% heat-inactivated FBS and 1% antibiotics. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2, trypsinized with a (0.05% trypsin–0.02% EDTA) solution, and passaged twice a week.

Tetracosanoic acid (C24:0) and hexacosanoic acid (C26:0) (Sigma–Aldrich) were solubilized in α-cyclodextrin (Sigma–Aldrich) as previously described (Singh and Kishimoto, 1983).