Molecular Genetics and Metabolism 106 (2012) 231–236

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

The effect of bone marrow transplantation on oxidative stress in X-linked adrenoleukodystrophy

Francieli J. Rockenbach [a], Marion Deon [a,b,*], Daiane P. Marchese [b], Vanusa Manfredini [e], Caroline Mescka [d], Graziela S. Ribas [a,b], Clarissa T. Habekost [b], Claudio G. Castro Jr. [c], Laura B. Jardim [b], Carmen R. Vargas [a,b,d,*]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, UFRGS, Porto Alegre, RS, Brazil
[b] Serviço de Genética Médica, HCPA, Porto Alegre, RS, Brazil
[c] Serviço de Oncologia Pediátrica, HCPA, Porto Alegre, RS, Brazil
[d] Programa de Pós-Graduação em Ciências Biológicas, Bioquímica, UFRGS, Porto Alegre, RS, Brazil
[e] Programa de Pós-Graduação em Bioquímica, UNIPAMPA, Uruguaiana, RS, Brazil

ARTICLE INFO

Article history:
Received 12 February 2012
Received in revised form 26 March 2012
Accepted 26 March 2012
Available online 3 April 2012

Keywords:
Bone marrow transplantation
Adrenoleukodystrophy
Oxidative stress
Free radicals
Lipid peroxidation
Protein damage

ABSTRACT

Oxidative stress plays an important role in the pathophysiology of neurodegenerative diseases, including X-linked adrenoleukodystrophy (X-ALD). In the present work, we evaluated lipid (malondialdehyde [MDA] content) and protein (sulfhydryl and carbonyl contents) oxidative damage parameters in plasma from X-ALD patients before and after bone marrow transplant (BMT), in order to verify if this treatment is capable to alter the oxidative parameters studied. We also evaluated the plasma concentration of hexacosanoic acid (C26:0) from X-ALD patients and correlated it with the oxidative damage parameters investigated. We observed that MDA content was significantly increased in plasma of X-ALD patients before BMT and after BMT when compared to controls, and that it was significantly reduced in plasma of X-ALD after BMT when compared to the before BMT group. These results indicate that lipid peroxidation is stimulated in X-ALD patients but there is a significant reduction of lipid peroxidation after BMT. Next, we observed a significant reduction of sulfhydryl content in plasma of X-ALD patients before BMT compared to controls indicating protein oxidative damage and that this measurement was increased in these patients after BMT as compared to before BMT. We found no significant differences in plasma carbonyl content in X-ALD patients before and after BMT as compared to controls. However, we observed a significant reduction in this parameter in X-ALD patients after BMT compared to before BMT. Finally, C26:0 plasma concentration was significantly reduced in X-ALD patients after BMT when compared to before BMT. We found no significant correlations between MDA and carbonyl values with C26:0 levels of the patients before BMT and after BMT, but a significant inverse correlation between sulfhydryl content and C26:0 levels was detected. In conclusion, the present study reinforces the hypothesis that lipid peroxidation and protein damage are induced in plasma of X-ALD patients and, in addition, demonstrates that BMT treatment is capable to reduce this pathogenic process. Taken together, the data obtained from plasma of X-ALD patients before and after BMT showing induction and protection, respectively, of oxidative stress, allowed to suggest that BMT, when well succeeded and under the recommendations, is effective to reduce C26:0 plasma levels and the increased lipid and protein oxidative damage in X-ALD.

1. Introduction

X-linked adrenoleukodystrophy (X-ALD) is the most frequent peroxisomal disorder with an estimated frequency of 1:21000 males that involves mainly the white matter and axons of the central nervous system (CNS), the adrenal cortex and testis [1,2]. Biochemically, this disease is characterized by the accumulation of very long chain fatty acids (VLCFA) particularly hexacosanoic acid (C26:0) and tetracosanoic acid (C24:0) in tissues and body fluids [3,4].

X-ALD presents a wide range of phenotypic variability sharing the same defective gene ABCD1 located within the Xq28 region that belongs to ATP-binding cassette (ABC) superfamily of transmembrane transporters and encodes the ALD protein (ALDP) which is located in the peroxisomal membrane [2,5,6]. It is a heterogeneous disease with seven different phenotypes in male patients being that the childhood cerebral form (CCER) and the adrenomyeloneuropathy (AMN) are the most prevalent and with five phenotypes in female carriers — heterozygotes [2,7].

* Corresponding authors at: Serviço de Genética Médica, HCPA, Rua Ramiro Barcelos, 2350, CEP 90.035-003, Porto Alegre, RS, Brazil. Fax: +55 51 33598010.
E-mail addresses: marion_deon@yahoo.com.br (M. Deon), crvargas@hcpa.ufrgs.br (C.R. Vargas).

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doi:10.1016/j.ymgme.2012.03.019

The CCER form manifests symptoms usually from age 4 to 8 years, including visual and auditory disturbances, decreased school performance, adrenal insufficiency, walking difficulties, demyelination and leukodystrophy, which seem to be associated with a strong inflammatory reaction in the CNS, particularly in the white matter. The disease progresses rapidly and patients usually die approximately 2 to 5 years after symptom onset [7].

Current treatment options for X-ALD is limited on three modes of therapy and can change as the phenotypes evolve: adrenal hormone replacement, Lorenzo's Oil (LO) therapy and bone marrow transplant (BMT) or hematopoietic stem cell transplantation (HSCT) [8,9,7].

So far, bone marrow transplant (BMT) or hematopoietic stem cell transplantation (HSCT) have been the only known method to halt cerebral demyelination [2,7,9]. The first successful transplant took place in 1990 on an eight-year-old boy reversing his neurological and neuroradiological manifestations and restoring his cognitive functions to normal despite mildly raised levels of VLCFA [5]. In general, it is only recommended for individuals with mild evidence of brain involvement by magnetic resonance imaging (MRI) but minimal neuropsychological findings (performance Intelligence Quotient (IQ) >80) and normal clinical neurologic examination, because lower IQ levels are related with BMT complications and early death. Further, boys with few neurological findings (MRI Loes Score <10) have a survival probability of 92% after HSCT and seem to have a better long-term neurological outcome [10–12]. The methods is an option for boys and adolescents who are in early stage of cerebral involvement and can provide long-term stabilization and even reversal of symptoms. BMT is not recommended for individuals with severe neurologic and neuropsychological dysfunction (i.e., performance IQ <80) [10,11,13–16].

The lack of effective and curative treatment for X-ALD may probably reflect the fact that the pathophysiology of the brain injury in this disorder is poorly known. In this context, oxidative stress is believed to be an important mediator of neurodegeneration since the CNS is highly susceptible to oxidative damage due to the relatively low activity of antioxidant defenses, high iron content, high lipid content, specially unsaturated fatty acids, and high oxygen consumption [17]. Preliminary results from our laboratory showed a significant increase in lipid peroxidation and a decreased antioxidant defense in symptomatic and asymptomatic X-ALD patients [18–21]. These results are in accordance with some studies in vitro and in animal models of X-ALD (knockout mice ABCD1) in which increased oxidative stress has been reported [22–24]. In addition, it has been reported that antioxidants (like vitamin E) reverses the oxidative damage in fibroblasts from X-ALD patients [24].

In the present work, we aimed to evaluate lipid (malondialdehyde content) and protein (sulfhydryl and carbonyl contents) oxidative damage parameters in plasma from X-ALD patients before and after BMT, in order to verify if this treatment is capable to alter the oxidative parameters studied. We also evaluated the plasma concentration of C26:0 from X-ALD patients before and after BMT and correlated plasma C26:0 levels with the oxidative damage parameters investigated.

2. Materials and methods

2.1. Patients and controls

In the present study we evaluated different parameters of oxidative stress in plasma from 4 X-ALD patients before BMT (at a "basal time": diagnosis moment or without any treatment for X-ALD) and after BMT (6 months to 4 years after BMT). The ages and main clinical features of X-ALD patients before and after BMT, as well as other characteristics (donor type, outcome, MRI findings) are presented in Table 1.

The diagnosis of X-ALD was established when increased concentrations of very long chain fatty acids (VLCFA), such as hexacosanoic acid (C26:0), tetracosanoic acid (C24:0) and the ratio C26:0/C22:0 and C24:0/C22:0 were found in plasma [3,25]. The measurements of VLCFA plasma levels at diagnosis and after BMT were performed by gas chromatography in Medical Genetics Service of the Clinical Hospital of Porto Alegre, RS, Brazil [25]. The control group consisted of healthy male individuals with similar ages to the patients (6.33 ± 1.87 years old, range 4 to 9 years old).

The study was conducted according to the recommendations of the Ethics Committee of the Clinical Hospital of Porto Alegre. Parents of all patients and controls gave informed written consent to participate in the investigation.

2.2. Preparation of plasma

Plasma was separated from whole blood samples obtained from controls (healthy individuals) and from X-ALD patients before and after BMT by venous puncture with heparinized vials. Whole blood was centrifuged at 3000 rpm, plasma was rapidly removed by aspiration and frozen at −80 °C until analysis.

2.3. Malondialdehyde (MDA) determination

MDA was measured by high performance liquid chromatography (HPLC) following the method described by Esterbauer and Cheeseman [26], with some modifications. Briefly, 600 μL of trichloroacetic acid 28% and 1.4 mL of distilled water were added to 100 μL of human plasma. Addition of trichloroacetic acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds. Samples then were centrifugated at $1500 \times g$ for 5 min. After centrifugation, the supernatant was removed and MDA was separated by HPLC, using an amino-phase column analysis with mobile phase acetonitrile, 30 mM Tris buffer, pH 7.4 (1:9; v/v). The flow rate was 0.5 mL/min and the eluate was monitored at 267 nm, the absorption maximum of the enolate anion form of free MDA. The system was calibrated with a standard solution of MDA, which was used for quantification. Results were expressed in μM of MDA.

2.4. Sulfhydryl content

This assay is based on the reduction of 5.5-dithio-bis (2-nitrobenzoic acid) (DTNB) by thiols, which in turn become oxidized (disulfide), generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm, according to the method described by Aksenov and Markesbery [27]. The sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were reported as nmol TNB/mg protein.

2.5. Carbonyl content

Oxidatively modified proteins present an enhancement of carbonyl content [28]. In this paper, carbonyl content was assayed according to the method described by Levine et al. [29] based on the reaction of protein carbonyls with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm. The carbonyl content was calculated using a millimolar absorption coefficient of the hydrazone ($21{,}000\ M^{-1}\ cm^{-1}$). Values of carbonyl content were expressed as nmol carbonyl/mg protein.

2.6. Plasma C22:0, C24:0 and C26:0 quantification

Plasma docosanoic acid (C22:0), tetracosanoic acid (C24:0) and hexacosanoic acid (C26:0) were analyzed according to the technique of Moser and Moser that use gas chromatography [25]. This laboratorial procedure consisted of the preparation of total lipid extract and after a treatment of this extract with methanolic HCl (3 N) for the formation of fatty acid methyl esters, which were then purified by thin-layer chromatography. The fatty acid methyl esters purified were extracted with hexane and analyzed by gas chromatography. A Varian gas