Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 827 (2005) 76–82
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits

Raquel Mateos, Elena Lecumberri, Sonia Ramos, Luis Goya, Laura Bravo *

Instituto del Frío (CSIC), C/José Antonio Novais 10, Ciudad Universitaria, E-28040-Madrid, Spain

Received 8 February 2005; accepted 9 June 2005
Available online 11 July 2005

Abstract

A high-performance liquid chromatography (HPLC) method to determine malondialdehyde (MDA) as the 2,4-dinitrophenylhydrazine (DNPH) derivative was applied to biological samples (serum and liver homogenates). Since MDA is considered a presumptive biomarker for lipid peroxidation in live organisms, a model for nutritionally induced oxidative stress (hypercholesterolemic rats) was studied in comparison with normocholesterolemic animals. The effect of diet supplementation with fruits rich in antioxidant polyphenols was assessed. The proposed method showed to be precise and reproducible, as well as sensitive enough to reflect differences in the oxidative status in vivo. A significant decrease of serum and liver MDA concentrations in animals fed diets containing 0.3% of polyphenols from strawberry, cocoa or plum was observed in the normocholesterolemic groups. This reduction was especially noteworthy in the hypercholesterolemic animals, with increased MDA levels indicating enhanced lipid peroxidation in the controls, yet with values parallel to the normocholesterolemic groups in animals fed the polyphenol-rich diets. These results point out the beneficial effects of phenolic antioxidants from fruits in preventing oxidative damage in vivo.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Malondialdehyde; HPLC; 2,4-Dinitrophenylhydrazine; Lipid peroxidation; Oxidative stress; Serum; Liver; Fruit antioxidant polyphenols

1. Introduction

Free radical mediated cytotoxicity and lipid peroxidation are associated with cell aging and chronic diseases such as cancer, atherosclerosis, inflammation, etc. [1–4]. Reactive oxygen species (ROS) can react with double bonds of polyunsaturated fatty acids (PUFAs) to yield lipid hydroperoxides. One of the major secondary oxidation products of peroxidized polyunsaturated fatty acids is malondialdehyde (MDA) [5,6], which has been inferred to have mutagenic and cytotoxic effects, and possibly to participate in the onset of atherosclerosis [7]. Since MDA has been found elevated in various diseases thought to be related to free radical damage, determination of this biomarker has been widely applied as the most common approach for the assessment of lipoperoxidation in biological and medical sciences [7,8]. MDA is also widely used in food sciences as an index of lipid oxidation and rancidity in foods and food products [9].

MDA is most frequently determined spectrophotometrically as thiobarbituric acid reactive substances (TBARS) after its reaction with thiobarbituric acid (TBA) at 100 °C in acidic media and measuring absorbance of the reaction mixture at 532 nm [8]. This is a simple and inexpensive method, yet highly inaccurate since TBA reacts not only with MDA but also with many other compounds (e.g. carbohydrates, pigments, amino acids, pyridins, etc.) [9,10], interfering in the TBA assay and resulting in considerable vari-

* Corresponding author. Tel.: +34 915445607; fax: +34 915493627.
E-mail address: lbravo@if.csic.es (L. Bravo).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.06.035

ability in the results. On the other hand, its chromatographic approach measures the 2TBA–MDA adduct after separation by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence [6,11–13] or UV–vis detection at 532 nm [7,14]. However, reaction of MDA with TBA still requires treatment at high temperatures (95–100 °C) for extended incubation times (up to 150 min) [6,11–14] in strong acidic conditions (pH 1.5–3.5), which may result in an artefactual peroxidation of sample constituents even in the presence of added antioxidants (i.e. BHT). In spite of this, TBARS is still widely used to determine MDA levels in human [15–19] and animal experiments [20–26], measuring MDA in biological samples such as plasma, or organs like liver, lung or kidney.

Derivatisation of MDA and other aldehydes with pentafluorophenylhydrazine and separation by GC–MS has been applied to foods as well as biological samples with good results [27–29], allowing the simultaneous determination of several aldehydes, although this method requires the use of expensive equipment. Alternatively, derivatisation of MDA with 2,4-dinitrophenylhydrazine (DNPH) and conversion into pyrazole and hydrazone derivatives has been found to allow specific estimation of this compound, especially if combined with its separation using HPLC [30,31], a technique now commonly available in research laboratories. This approach has been used to determine MDA levels in biological samples, such as rat and human plasma [5,31,32] or rat urine [30], although it has not been applied yet to tissue samples such as liver or lung.

In a previous work [33], we reported a methodology to determine MDA by HPLC as the 2,4-dinitrophenylhydrazone derivative in human hepatoma HepG2 cells in culture. This showed to be an accurate, sensitive and reproducible method that reflected the effect of induced oxidative stress on the levels of lipid peroxidation in cells, suggesting that it could be used as a reliable biomarker for cellular oxidative stress. The aim of this study, was to adapt this method to biological samples, such as serum and liver homogenates, and to test its feasibility as a biomarker for oxidative stress in vivo in a rat model for hypercholesterolemia.

2. Experimental

2.1. Materials

Acetonitrile, methanol, sodium hydroxide, sodium chloride, di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, as well as formic, hydrochloric, perchloric and sulphuric acids were acquired from Panreac (Barcelona, Spain). Trizma base, sucrose, DL-dithiotreitol (DTT), 2,4-dinitrophenylhydrazine and 1,1,3,3-tetraethoxypropane (TEP) were purchased from Sigma Chemical Co. (Madrid, Spain). All reagents were of analytical grade unless stated otherwise.

Table 1
Composition of the experimental diets (g/kg dry weight)[a]

	Control	Strawberry	Cocoa	Plum
Casein	140	140	140	140
Dextrose	155	155	155	155
Sucrose	100	0	100	0
Soya oil	40	40	40	40
BHQ[b]	0.008	0.008	0.008	0.008
Mineral mix	35	35	35	35
Vitamin mix	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Corn starch	465.692	460.692	350.692	460.692
Choline bitartrate	2.5	2.5	2.5	2.5
Cellulose	50	35	0	35
PP-rich ingredient	–	120	165	120
Total	1000.00	1000.00	1000.00	1000.00

[a] Hypercholesterolemic diets were supplemented with 20 g cholesterol and 4 g sodium cholate per kg of diet, at the expense of starch.
[b] *tert*-Butylhydroquinone.

2.2. Animal experiment

Male Wistar rats (8 weeks old) were obtained from the School of Medicine, Universidad Complutense (Madrid, Spain). They were placed individually in metabolic cages and housed in a room under controlled conditions of temperature (19–23 °C), humidity (50–60%) and light (12 h light/12 h dark cycles). Rats were randomly assigned to the different dietary groups (eight groups, eight animals per group). The composition of the diets is given in Table 1.

Normocholesterolemic diets were prepared from a Fiber Free AIN-93M Purified Rodent Diet (Panlab S.L., Barcelona, Spain), which provides the nutrients required by adult rats according to the National Research Council guidelines [34,35]. Hypercholesterolemic diets were prepared from the AIN-93M diet supplemented with 20 g of cholesterol and 4 g of sodium cholate per kilogram of the basal diet [36]. Cellulose (5%) was added to the basal fibre free diet and fed to the control groups. The experimental diets were prepared from the fibre free basal diet and contained 12% of freeze-dried strawberry or plum powder, or 16.5% of cocoa fibre. These powdered fruit supplements were added as a source of dietary fibre and polyphenols at the expense of starch. Diets were formulated to provide 3 g of polyphenols per kg of diets. Cellulose was added to the strawberry and plum diets to make up for a final 5% dietary fibre. Since both strawberry and plum powders were rich in soluble sugars, sucrose was not added to the corresponding diets (Table 1). Caloric content was similar in all four diets.

Animals were adapted to the powdered diets for four days prior to the experimental period that lasted three weeks. During this time, rats were fed the corresponding normo or hypercholesterolemic diets. Animals were given free access to food and water. Body weight and food intake were monitored daily throughout the experiment. After the experimental period, rats were sacrificed, troncal blood collected, and serum separated by centrifugation (3000 rpm, 10 min, 4 °C) and kept

Download English Version:

https://daneshyari.com/en/article/10550145

Download Persian Version:

https://daneshyari.com/article/10550145

Daneshyari.com