Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 40 (2017) 95–104

Journal of Nutritional Biochemistry

Antioxidant properties of tea blunt ROS-dependent lipogenesis: beneficial effect on hepatic steatosis in a high fat-high sucrose diet NAFLD obese rat model

Laura Braud[a,*], Sylvain Battault[b], Grégory Meyer[b], Alessandro Nascimento[b], Sandrine Gaillard[c], Georges de Sousa[d], Roger Rahmani[d], Catherine Riva[b], Martine Armand[e], Jean-Michel Maixent[a,1], Cyril Reboul[b,1]

[a]Laboratoire Protee, Equipe EB2M, Université de Toulon, avenue de l'Université, 83130 La Garde, France
[b]Laboratoire de Pharm-Ecologie Cardiovasculaire (EA4278), Faculty of Sciences, Avignon University, 33 rue Louis Pasteur, 84000 Avignon, France
[c]Plateforme BioTechServices, Université de Toulon, avenue de l'Université, 83130 La Garde, France
[d]UMR 1331 TOXALIM (Research Centre in Food Toxicology), Institut National de la Recherche Agronomique (INRA), Laboratory of Xenobiotic's Cellular and Molecular Toxicology, 400 route des Chappes, 06903 Sophia-Antipolis, France
[e]Aix-Marseille Université, CNRS, CRMBM UMR 7339, F-13385 Marseille, France

Received 28 April 2016; received in revised form 19 September 2016; accepted 5 October 2016

Abstract

Oxidative stress could trigger lipid accumulation in liver and thus hepatic steatosis. Tea is able to prevent liver disorders, but a direct link between antioxidant capacities and prevention of steatosis has not been reported yet. We aimed to investigate such relationship in a rat model of high fat-high sucrose diet (HFS)-induced obesity and to explore more deeply the mechanisms in isolated hepatocytes. Wistar rats were divided into a control group (standard diet), an HFS group (high fat-sucrose diet) and an HFS + tea group (HFS diet with *ad-libitum* access to tea drink). Body weight, fat mass, glycemic parameters in blood, lipid and oxidative stress parameters in blood and liver were measured in each group after 14 weeks. Isolated hepatocytes were treated with the reactive oxygen species (ROS) inducer *t*-BHP in the presence or not of antioxidants (tempol or tea), and superoxide anion production and lipid accumulation were measured using specific fluorescent probes. We reported that the HFS diet highly increased hepatic lipids content, while tea consumption attenuated steatosis and improved the oxidative status (decrease in hepatic oxidative stress, increase in plasma total antioxidant capacity). The role of antioxidant properties of tea in such phenomenon was confirmed in primary cultured rat hepatocytes. Indeed, the increase of mitochondrial ROS production with *t*-BHP resulted in lipid accumulation in hepatocytes (positive linear regression), and antioxidants (tempol or tea) normalized both. We reported that the antioxidant properties of tea protect rats from an obesogenic HFS diet-induced hepatic steatosis by counteracting the ROS-dependent lipogenesis.
© 2016 Elsevier Inc. All rights reserved.

Keywords: NAFLD; *Camellia sinensis*; Antilipogenesis; Antioxidant; Mitochondrial ROS; Obesogenic diet

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a spectrum of chronic liver diseases ranging from hepatic steatosis to nonalcoholic steatohepatitis progressing to fibrosis and ultimately to cirrhosis [1]. Since NAFLD has been strongly associated with obesity, insulin resistance, hypertension and dyslipidemia and is now considered as the liver metabolic syndrome manifestation, it represents nowadays the most common and emerging chronic liver disease [2]. Because of the difficulty of the diagnosis, the exact global incidence of NAFLD is unknown. However, it has been estimated in western countries that the prevalence of NAFLD is about 30% while it is about 5–18% in Asia [3]. In obese subjects, NAFLD is characterized by the accumulation of lipids in the liver which is considered as the "first hit" in the progression from steatosis to steatohepatitis. The "second hit" is dependent on increased oxidative stress and characterized by lipid peroxidation, inflammation, mitochondrial dysfunction and hepatic injury [4]. Unfortunately, today, there are only limited options for the treatment of NAFLD, including lifestyle modification, weight management, vitamin E and thioglitazone treatment [5,6].

Epidemiological and clinical studies have shown that tea consumption was associated with decrease liver disorders [7,8]. But the underlying mechanisms associated with the hepato-protective effects of tea are still not clear. Indeed, tea is recognized for its antioxidant properties, mainly due to tea polyphenols, which contribute to protect ob/ob mice from hepatic steatosis [9]. The beneficial effects of tea are also reported to be due to direct antilipogenesis properties [10,11]. However, despite the described role of oxidative stress in hepatic lipogenesis [12–14], the contribution of the antioxidant properties of tea to its antilipogenesis action has never been investigated.

* Corresponding author at: Laboratoire PROTEE, EB2M, Université de Toulon, avenue de l'Université, 83130 La Garde, France. Tel.: +33-04-94-14-26-70; fax: +33-04-94-14-20-45.
E-mail address: lbraud01@gmail.com (L. Braud).
[1] Senior co-authors.

http://dx.doi.org/10.1016/j.jnutbio.2016.10.012
0955-2863/© 2016 Elsevier Inc. All rights reserved.

The aim of this work was to test whether the antioxidant properties of tea could be in part responsible of its antilipogenesis effects. For this purpose, we used an obesogenic high-fat–high-sucrose diet in rats to examine the effect of tea on hepatic lipid parameters, antioxidant capacity and lipid peroxidation, together with a primary culture of rat hepatocytes to deeply explore the underlying mechanisms.

2. Materials and methods

2.1. Animals and experimental design

Seven-week-old male Wistar rats weighing about 200 g (±25 g) were obtained from Janvier (France). Three rats were housed per cage under controlled conditions of temperature (21 °C±1), hygrometry (60% ± 10) and lighting (12 h a day). Animals were acclimatized to the animal laboratory for 7 days before the start of the experiment. Rats were randomly divided into three groups: control group, high-fat–high-sucrose diet (HFS) group and HFS + tea group (n=15 per group). The control group was fed with a standard diet while the others consumed high-fat diet (60% fat Purified Diet 230 HF). Standard diet (A04) and high-fat diet (Purified Diet 230 HF) were purchased from SAFE Diet (Augy, France). Sucrose was purchased from Sigma Chemical Co. (St Louis, MO, USA) and supplied at the dose of 10% in the drink for the HFS groups. Tea infusion was supplied as drink for the HFS + tea group. Rats were provided free access to food and fluid for 14 weeks, and dietary consumptions were recorded three times each week. Consumption of tea was about 50 mL per day per rat. The composition of experimental diets is shown in Table 1. All along the protocol rats were weighed weekly. Rats were sacrificed at the end of 14 weeks and after a 12-h fast. Blood was collected for biochemistry analysis. Total dorsal and epididymal fat were removed and weighed as index of visceral obesity. Livers were removed in order to perform biochemical measurements. All animals received care according to institutional guidelines, and all experiments were approved by the institutional ethics committee (Agreement number A-84-007-2).

2.2. Tea infusion characterization and preparation

Hao Ling® tea is a blend of green, oolong and pu-erh tea leaves coming from Zhejiang, Fujian and Yunnan province (China), respectively. This blend of teas is elaborated following a confidential manufacturing process by the french company "Thés de la Pagode" (Paris). The infusions were prepared by pouring 200 mL of distilled water at 100 °C on 20 g of tea leaves mixture powder and brewing for 15 min. The tea infusion obtained was then diluted in 4 volumes of tap water to obtain a tea infusion at human nutritional doses. For culture cell experiments, the infusion was lyophilized using HetoPowerDry LL1500 (Thermo Electron, USA), and the freeze-dried aqueous tea extracts was stored at −20 °C until analysis. Total phenolic content and quantification of aqueous tea extracts compounds were performed as previously described [15]. In the diluted aqueous tea infusion, total polyphenols, catechins, gallic acid and caffeine content were 126.6 mg, 10.7 mg, 8.4 mg and 41.12 mg per 100 mL (Table 2), respectively. The amount of the main catechins, epigallocatechin, epicatechin, epigallocatechin gallate and epicatechin gallate were 1.2 mg, 2.2 mg, 5.5 mg and 1.8 mg per 100 mL of aqueous tea infusion, respectively (Table 2).

2.3. Fasting blood glucose, glucose and insulin tolerance tests

An Oral Glucose Tolerance Test (OGTT) was performed at the end of the 13th week of the chronic protocol. First, blood was obtained *via* tail clip to assess fasting blood glucose (Caresens® N, DinnoSanteTM). Then, rats received a glucose solution (2 g/kg) by oral gavage, and blood glucose was measured at 20, 40, 60, 80, 100 and 120 min after the glucose oral gavage. On the following week, intraperitoneal Insulin Tolerance Test (ITT) was performed. First, fasting blood glucose was measured, and then, rats received an intraperitoneal injection of insulin solution (2 UI/kg), and blood glucose was measured at 30, 60, 90, 120, 150, 180 and 210 min after insulin injection.

Table 1
Experimental diets composition

Composition	Control	HFS	HFS + Tea
Lipids (%)	3.1	35.8	35.8
Proteins (%)	16.1	17.4	17.4
Carbohydrates (%)	3.37	35	35
Crude fibers (%)	3.9	0	0
Minerals (%)	4.5	5.8	5.8
NFE (%)	59.9	33.3	33.3
Vitamin A (UI/kg)	6600	20,000	20,000
Vitamin D3 (UI/kg)	900	2500	2500
Vitamin E (UI/kg)	30	175	175

NFE (nitrogen-free extract) = 100 - (H_2O) - (protein) - (fat) - (mineral) - (crude fiber).

Table 2
Total polyphenol, total catechin, gallic acid and caffeine content (mg/100 ml) of tea infusion given to the rats

Compound structures	mg/100 ml of aqueous infusion [1]
Total polyphenol	126.6±0.63
Total catechin	10.7±0.56
Epigallocatechin	1.2±0.13
Epicatechin	2.2±0.13
Epigallocatechin gallate	5.5±0.19
Epicatechin gallate	1.8±0.13
Gallic acid (GA)	8.4±0.19
Caffeine	41.12±0.31

The tea infusions were prepared by pouring 200 mL of distilled water at 100 °C on 20 g of tea leaves mixture powder and brewing for 15 min. The given tea infusion obtained was then diluted in 4 volumes of tap water.
[1] Results are expressed as means ± S.E.M. of three parallel measurements (n=3).

2.4. Analysis of plasma lipids and ALT concentrations

Enzymatic assays (kits from Roche, Indianapolis, USA) were performed on Cobas 6000 analyzer (Roche) for measuring triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and alanine transaminase (ALT). Nonesterified fatty acid (NEFA) was performed with Randox kit (Randox Laboratories, UK).

2.5. Measurement of liver lipid content

Portions of liver samples from each group of rats were weighed and homogenized in phosphate buffered saline (PBS) (0.01-M phosphate, 0.0027-M potassium chloride, 0.137-M sodium chloride at pH 7) using a IKA T10 basic ULTRA TURRAX Homogenizer (Sigma-Aldrich). Lipid extraction from homogenates was performed using a mixture of chloroform/methanol 2/1 v,v [16], and contents of triglycerides and of the total cholesterol (PAP 150 kit and RTU kit, respectively, from Biomérieux, Lyon, France) and of NEFA (Randox kit, Randox Laboratories, United-Kingdom) were quantified.

2.6. Analysis of mRNA expression

After an initial extraction step by mixing Extract-All (Eurobio, France) and chloroform to samples, hepatocytes' total RNA purification was performed with a column extraction Kit (RNeasy Mini®, Qiagen, Germany). A supplementary rDNase treatment was added to the manufacturer's instructions in order to remove any genomic DNA contamination. This one was excluded by a control PCR amplification of RNA. Double-strand cDNA was synthesized from total RNA according to the manufacturer's manual (Omniscript®, Qiagen, Germany). SYBR Green real-time PCR quantifications were performed using commercially available RT^2 Profiler™ PCR Arrays from Qiagen (Germany) containing primer pairs that have been experimentally validated to ensure gene-specificity and high amplification efficiency on a LightCycler 480 (Roche, Germany). Levels of mRNA, expressed as relative mRNA levels compared with control diet-fed rats, were calculated after normalization to RPL17 reference gene.

2.7. Hepatic lipid peroxidation and antioxidant capacity

The hepatic level of lipid peroxidation expressed as hepatic malondialdehyde (MDA) equivalent was evaluated using QuantiChrom Thiobarbituric acid reactive substances (TBARS) and the Glutathione Peroxidase (GPx) activity was measured with GPx assay kits from BioAssay Systems (USA). Total hepatic antioxidant capacity was assessed using Antioxidant Assay Kit from Sigma Chemical Co. (St Louis, MO, USA). Total antioxidant capacity of plasma was determined using OxiSelect™ TAC assay kit from Cell BioLabs (USA).

2.8. Measurement of lipid accumulation and mitochondrial superoxide anion on primary culture of rat hepatocytes

Hepatocytes were freshly isolated from *Rattus norvegicus* ofa male rats weighing 180 to 200 g (Iffa Credo, France) by collagenase perfusion of the liver [17]. The national guidelines for care and use of research animals were followed (agreement number A 13823, French Ministry of Agriculture). Hepatocytes were seeded as previously described [15]. Cells were then treated for 12 h with 100 µL of various concentrations of aqueous tea extract (100, 250 and 500 µg/mL) with or without 120 µM of exogenous oxidative stress inducer *t*-BHP (*tert*-butyl hydroperoxide in DMSO solution, both purchased from Sigma-Aldrich, France). Such doses were selected after testing several concentrations (from 100 µM to 1 mM) in order to generate sufficient oxidative stress without killing all cells (data not shown). The relative hepatocyte lipid accumulation and mitochondrial superoxide anion were measured using Neutral Lipid Stains (LipidTox) green staining and mitochondrial superoxide indicator (MitoSox) red staining probes (Life Technologies, France), respectively, as previously described [15]. Control, that is, "non-treated" cells, consisted of medium, water or DMSO depending on

Download English Version:

https://daneshyari.com/en/article/5512921

Download Persian Version:

https://daneshyari.com/article/5512921

Daneshyari.com