Steroids xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Steroids

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

Increased flux of the plant sterols campesterol and sitosterol across a disrupted blood brain barrier

Ahmed A. Saeed [a,b], Guillem Genové [c], Tian Li [c], Frank Hülshorst [d], Christer Betsholtz [c], Ingemar Björkhem [a], Dieter Lütjohann [d,*]

[a] Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institute Huddinge, 14186 Huddinge, Sweden
[b] Department of Biochemistry, Faculty of Medicine, University of Khartoum, 11111 Khartoum, Sudan
[c] Department of Medical Biochemistry and Biophysics, Karolinska Institute, 17177 Stockholm, Sweden
[d] Institute for Clinical Chemistry and Clinical Pharmacology, University Clinics Bonn, 53105 Bonn, Germany

ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form 27 January 2015
Accepted 3 February 2015
Available online xxxx

Keywords:
Phytosterols
Stable isotopes
Blood–Brain-Barrier
Brain lipids
Cholesterol metabolism
Plant sterols

ABSTRACT

The intact blood–brain barrier in mammalians prevents exchange of cholesterol loaden particles between periphery and brain and thus nearly all cholesterol in this organ originates from *de novo* synthesis. Dietary cholesterol homologues from plants, campesterol and sitosterol, are known to get enriched to some extent in the mammalian brain. We recently showed that *Pdgfb*$^{ret/ret}$ mice, with a pericyte deficiency and a leaking blood–brain barrier phenotype, have significantly higher levels of plant sterols in the brain compared to their heterozygous *Pdgfb*$^{ret/+}$ controls keeping the integrity of the blood–brain barrier (BBB). In order to further study the protective functionality of the BBB we synthesized a mixture of [$^2H_6$]campesterol/sitosterol and fed it for 10–40 days to genetically different types of animals. There was a significant enrichment of both deuterium stable isotope labeled plant sterols in the brain of both strains of mice, however, with a lower enrichment in the controls. As expected, the percentage and absolute enrichment was higher for [$^2H_6$]campesterol than for the more lipophilic [$^2H_6$]sitosterol. The results confirm that a leaking BBB causes increased flux of plant sterols into the brain. The significant flux of the labeled plant sterols into the brain of the control mice illustrates that the presence of an alkyl group in the 24-position of the steroid side chain markedly increases the ability of cholesterol to pass an intact BBB. We discuss the possibility that there is a specific transport mechanism involved in the flux of alkylated cholesterol species across the BBB.

1. Introduction

Plant sterols, also called phytosterols, are naturally occurring sterols in the plants. They cannot be synthesized by mammalian cells and can only be derived from dietary sources. Campesterol and sitosterols are two of the most prevalent phytosterols. Structurally they differ from cholesterol only by an extra methyl group (campesterol) or ethyl group (sitosterol) at position C-24 [1].

The mammalian blood–brain barrier (BBB) is not permeable for cholesterol and there is no flux of lipoprotein-bound cholesterol from the circulation into the brain. Surprisingly, both campesterol and sitosterol have been shown to cross the blood–brain barrier to some extent. Vanmierlo et al. showed that mice fed with a plant sterol enriched diet had higher levels of both compounds in the brain than the controls [2]. In another study, mice with abcg5 or abcg8 deficiency and high levels of plant sterols in the circulation were shown to have several-fold higher levels of campesterol and sitosterol in the brain than the wild type [3].

We recently reported that disruption of BBB function in *Pgdfb*$^{ret/ret}$ mice results in a significant flux of cholesterol from the circulation into the brain [4]. These mice were shown to have significantly higher levels of campesterol and sitosterol in the brain than the controls, suggesting that BBB is of some importance for the flux of plant sterols into the brain.

In the present work we further investigated the role of the BBB for the flux of plant sterols between brain and circulation by feeding mutated mice and their controls with a diet enriched with deuterium labeled phytosterols. Our results suggest that BBB has a protective function against crossing of phytosterols. However, this function is considerably less effective than in the case of cholesterol.

* Corresponding author at: Institute for Clinical Chemistry and Clinical Pharmacology, University Clinics Bonn, Sigmund-Freud-Str. 25, D-53105 Bonn, Germany. Tel.: +49 (0) 228 2871 4027; fax: +49 (0) 228 2871 6047.
E-mail address: dieter.luetjohann@ukb.uni-bonn.de (D. Lütjohann).

http://dx.doi.org/10.1016/j.steroids.2015.02.005
0039-128X/

2. Methods

2.1. Animals and tissues

Transgenic mice (*Pgdfb*$^{ret/ret}$) that lack the retention motif of PDGF-B and their littermate controls (*Pgdfb*$^{ret/+}$) were used in this study [5]. The mice were housed under standard condition in enriched environment with 12 h light/dark cycle. The study was performed on 10 weeks old male mice. All experimental procedures in the animals were approved by Northern Stockholm Research Animal Ethics Committee.

To harvest the tissue, the mice were euthanized by carbon dioxide and blood was collected by heart puncture. The animals were then infused using ringer lactate to flush blood out from the organs. The two halves of the brain, liver, lungs, proximal intestine, testis and adrenals were collected. The tissue samples were immediately snap-frozen in liquid nitrogen and then stored at −80 °C until analysis. Plasma was separated from the whole blood and stored at −20 °C.

2.2. Synthesis of [2.2.3.4.6.6-$^2H_6$]phytosterols

A plant sterol mixture (brassicasterol/campesterol/stigmasterol/sitosterol; 10/42.7/0.3/47.0; Fig. 1; 1) is oxidized in the presence of aluminum tert.-butoxide and acetone to the corresponding side-chain heterologous cholest-4-ene-3-one derivative 2, which is deuterated with sodium methylate and CH_3OD to the [2.2.4.6.6-$^2H_5$]enone 3. After chromatographic purification compound 3 is suspended in iso-propenyl acetate and treated with 0.07 equivalents of concentrated D_2SO_4 and CH_3COONa to achieve a reisomerisation of the double bond, while the enol is quenched as the acetate 4. The resulting [2.2.4.6.6]$^2H_4$-derivative 4 is dissolved in absolute THF and $^2H_2$-(D_2)-hydrogenated in a mixture of CH_3OD, C_2H_5OD, and D_2O in presence of deuterated sodium borohydride ($NaBD_4$) to yield the [2.2.3.4.6.6-$^2H_6$]brassicasterol/campesterol/stigmasterol/sitosterol mixture 5. The overall yield of the whole synthesis was about 35 %. Combined gas chromatography–mass spectrometry of the trimethyl silyl ether derivative of the mixture demonstrated that the two products had retention times slightly shorter than unlabeled campesterol and sitosterol, respectively (as expected for a deuterated analogue). The mass spectra were in agreement with the proposed structure (Fig. 2). Thus the molecular ion of the derivative of campesterol had shifted from *m/z* 472 to 478 and the molecular ion of the derivative of sitosterol from *m/z* 486 to *m/z* 492. The percentage of deuterium in the two plant sterols was calculated by dividing the area of its corresponding peak in the tracing by the total area. The percentage isotopic distribution for one of the most intensive peak (Molecular ion minus OTMSi: [M^+] (−90)]) of the TMSi-ether of [2.2.3.4.6.6-$^2H_6$]campesterol (Fig. 2A): *m/z* 385.5, [M^+] (−3) (−90), 1.6%; *m/z* 386.5, [M^+] (−2) (−90), 10.3%; *m/z* 387.5, [M^+] (−1) (−90), 33.3%, *m/z* 388.5 [M^+] (−90), 41.5%; *m/z* 389.5, [M^+]+1 (−90) 11.6%; *m/z* 389.5, [M^+]+2 (−90) 1.7% and of [2.2.3.4.6.6-$^2H_6$]sitosterol (Fig. 2B): *m/z* 399.5, [M^+] (−3) (−90), 1.5%; *m/z* 400.5, [M^+] (−2) (−90), 10.7%; *m/z* 401.5, [M^+] (−1) (−90), 33.9%, *m/z* 402.5 [M^+] (−90), 41.0%; *m/z* 403.5, [M^+]+1 (−90) 11.2%; *m/z* 404.5, [M^+]+2 (−90) 1.7% (see Fig. 2).

2.3. Diet preparation

Chow diet from Harlem® was crushed into powder and mixed with deuterium-labeled phytosterol mixture (Figs. 1 and 5) dissolved in peanut oil. The final concentration of the oil and labeled phytosterols was 10% and 0.3%, respectively. In order to dissolve the sterols in the oil the mixture was heated at 60 °C for 10 min and then ultrasonicated. After mixing the oil thoroughly with the crushed chow it formed a homogenous paste.

2.4. Lipid extraction

The different organs were extracted with Folch's solution during 24 h at room temperature. Aliquots of this solvent were stored at −20 °C before analysis.

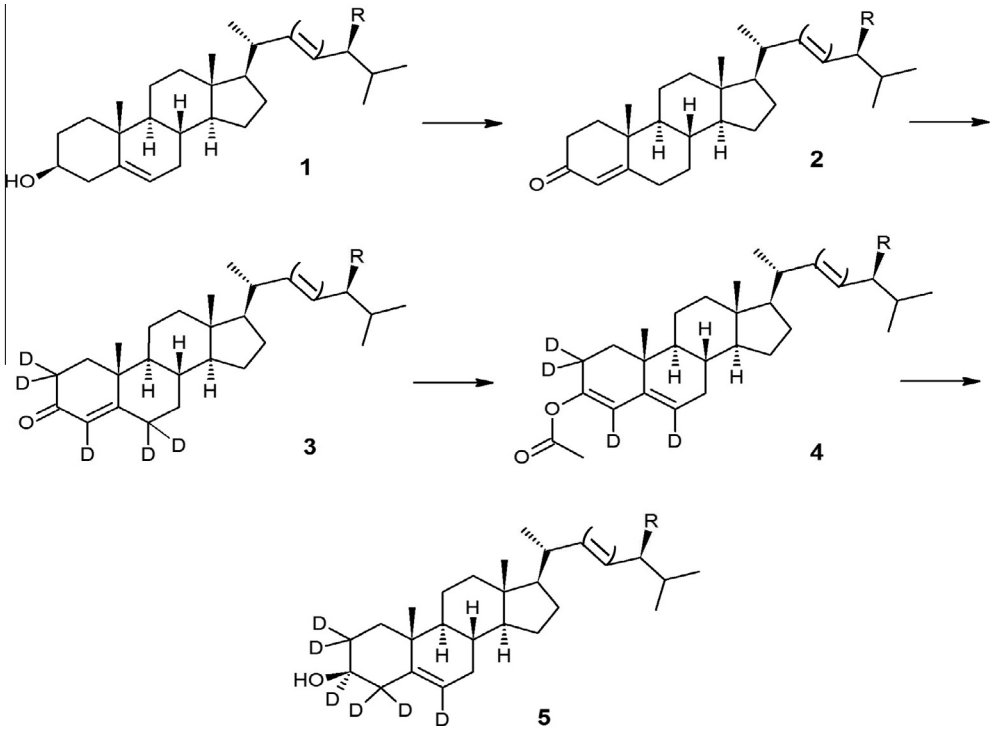

Fig. 1. Synthesis of [$^2H_6$]phytosterols. R = CH_3 = Brassicasterol (24-Methyl-cholest-4,22-dien-3β-ol); R = CH_3 = Campesterol (24-Methyl-cholest-4-en-3β-ol); R = C_2H_5-= Stigmasterol (24-Methyl-cholest-4,22-dien-3β-ol); R = C_2H_5-=Sitosterol (24-Methyl-cholest-4-en-3β-ol).

Please cite this article in press as: Saeed AA et al. Increased flux of the plant sterols campesterol and sitosterol across a disrupted blood brain barrier. Steroids (2015), http://dx.doi.org/10.1016/j.steroids.2015.02.005