Research Article

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Constitutive androstane receptor activation stimulates faecal bile acid excretion and reverse cholesterol transport in mice

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Background & Aims: The constitutive androstane receptor (CAR) is a nuclear receptor expressed in the liver and involved in xenobiotic metabolism. The aim of this study was to assess whether pharmacological CAR activation could affect neutral sterol and bile acid elimination under conditions of cholesterol overload.

Methods: Wild type, Car−/−, ApoE−/−, and low-density lipoprotein receptor (Ldlr)−/− mice fed a western-type diet were treated with the CAR agonist TCPOBOP.

Results: CAR activation was associated with a decrease in faecal cholesterol output related to the repression of the Abcg5/g8 cholesterol transporters. In contrast, TCPOBOP treatment induced a marked increase (up to three fold, p <0.01) in the elimination of faecal bile acids. In the liver, it was related to the coordinated induction of genes involved in synthesis, sulfo-conjugation, and excretion of bile acids as well as the repression of the ileal apical sodium-dependent bile acid transporter. Importantly, cholesterol accumulation was reduced in the liver of TCPOBOP-treated animals. In all cases, TCPOBOP had no effect in Car−/− mice. To determine directly whether CAR activation could affect the elimination of endogenous cholesterol, kinetic studies were performed with high-density lipoproteins (HDL) labelled with ^{3}H-cholesteryl esters. We observed that TCPOBOP-treated mice excreted more HDL cholesterol-derived bile acids in their faeces. Finally, long-term CAR activation was associated with decreases in cholesterol content of the whole body and atherosclerosis susceptibility.

Conclusions: CAR is involved in the control of cholesterol and bile acid homeostasis, increasing reverse cholesterol transport under hyperlipidemic conditions.

Keywords: Constitutive androstane receptor; TCPOBOP; Bile acids; High density lipoproteins; Cholesterol.

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Abbreviations: CAR, constitutive androstane receptor; TCPOBOP, 3,3′,5,5′-tetrachloro-1,4-bis(pyridyloxy)benzene; HDL, high density lipoproteins; CE, cholesteryl esters; LXR, liver X receptor.

Introduction

The nuclear receptor constitutive androstane receptor (CAR, NR1I3) is a xenobiotic sensor that controls the expression of genes involved in the hydroxylation, conjugation, and finally excretion of potentially harmful exogenous molecules [1–3]. In addition to these well-recognized functions, CAR plays a role in the metabolism of endogenous compounds such as bile acids, bilirubin, triglycerides, and thyroid hormones [2,4]. With regard to sterol metabolism, several independent studies have demonstrated that CAR is able to modulate plasma lipoprotein levels [5,6] and one of the well recognized functions of CAR is its ability to promote bile acid detoxification during cholestasis [7–11]. Unlike other nuclear receptors such as the Farnesoid X Receptor (FXR) or the Pregnane X Receptor (PXR), CAR does not seem to be directly activated by bile acids [1,8,11]. Nevertheless, it plays an important role by inducing the production of more hydrophilic and less hepatotoxic bile acids [7], both through stimulation of their conjugation [12] and activation of their excretion from hepatocytes through alternative export pathways [10,13]. The importance of CAR has been demonstrated *in vivo* in murine models of cholestasis (bile duct ligation) or in mice fed bile acid-enriched diets [7,8,10,11]. In these models, pharmacological CAR activation reduced bile acid toxicity [7,8,11] while, in contrast, CAR deficiency resulted in an exacerbation of liver damage [7,9,12]. Interestingly, CAR seems to exert its protective effects during cholestasis by stimulating bile acid elimination rather than turning off their synthesis [7]. Therefore, we wanted to investigate whether CAR activation could also modulate the elimination of cholesterol, *i.e.* the primary bile acid precursor, in mice fed a cholesterol-enriched diet. Indeed, biliary secretion of cholesterol and bile acids is a major pathway to remove excess cholesterol from the body. To address this question, WT mice, Car−/− mice and two hypercholesterolaemic mouse models (ApoE−/− and Ldlr−/− mice) were fed a western-type diet containing 0.15% cholesterol and treated weekly with the CAR specific agonist 3,3′,5,5′-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP) [14]. At the end of the treatment, the effect of CAR activation on sterol output as well as on expression of the main genes involved in bile acid and sterol metabolism was evaluated. We found that CAR activation resulted in a marked increase in faecal bile acid loss that was associated with a reduction in neutral sterol output.

By using plasma lipoproteins labelled with ^{3}H-cholesteryl esters, we observed that CAR activation was able to stimulate the elimination of cholesterol derived from HDL via its conversion into bile acids, *i.e.* the final step of the reverse cholesterol transport pathway. Altogether these data suggest that CAR activation is able to improve cholesterol homeostasis during diet-induced hypercholesterolaemia.

Materials and methods

A complete and detailed Material and methods section is available online in the Supporting information.

Animals

WT, Car−/− [3], Ldlr−/−, and ApoE−/− mice on a homogenous C57Bl6 background (Jackson laboratory, Bar Harbor, Maine) were used in the present study. TCPOBOP (Sigma–Aldrich, St. Louis, MO) was given at a dose of 3 mg/kg to the mice by a weekly intraperitoneal injection. The animals were fed a western-type diet (21% fat and 0.2% cholesterol, Safe, Augy, France or Diet 88137 (Harlan Tekland, Madison, WI). All experimental procedures were in accordance with the local guidelines for animal experimentation.

Reverse cholesterol transport

Mice were injected in the tail vein with high-density lipoproteins (HDL) labelled with ^{3}H-cholesteryl esters. Blood samples, livers, and faeces were collected up to 48 h after injection. Aliquots of the samples were used for total radioactivity determination or for selective extraction of neutral sterols and bile acids before scintillation counting.

Whole body cholesterol content determination

Cholesterol content was determined by an enzymatic method after digestion of the mouse bodies including the washed gastrointestinal tract in ethanol/KOH.

Atherosclerosis lesions

Percentage of the total surface area covered by atherosclerosis lesions was determined by en face analysis of aorta after staining with oil red O.

Real time PCR

Relative mRNA levels were determined by real time PCR using a Light-Cycler 2.0 (Roche, Meylan, France).

Western blot analysis

Total protein extracts in RIPA buffer (50 µg) were used for western blot analysis.

Sterol and bile acid concentration

Levels of neutral sterols and bile acids in the liver and the faeces were determined by capillary gas chromatography/mass spectrometry.

Results

TCPOBOP treatment reduces the faecal loss of cholesterol and increases bile acid output in WT but not Car−/− mice

WT and Car−/− mice fed a western-type diet were treated weekly with the CAR agonist TCPOBOP at 3 mg/kg. After 2 weeks of treatment, the faeces of individual animals were collected over a 24-h period and their neutral sterol and bile acid contents were determined. In WT mice, TCPOBOP treatment induced a decrease of the faecal cholesterol excretion but dramatically increased the faecal bile acid output with all the major bile species significantly higher in TCPOBOP-treated mice than in untreated mice (Fig. 1A). Interestingly, cholesterol concentration was significantly reduced in the livers of WT mice treated with TCPOBOP (Fig. 1C). It indicates that the decrease of faecal cholesterol excretion was not associated with hepatic cholesterol accumulation and suggests that cholesterol was efficiently removed from the liver at least in part via the bile acid pathway. As expected, TCPOBOP had no significant effect in Car−/− mice, neither on faecal sterol excretion nor on hepatic cholesterol concentration (Fig. 1B and D). Under a standard diet, a similar but less pronounced tendency was observed in TCPOBOP-treated-mice, with an increase in faecal bile acid excretion that was significant for beta- and alpha-muricholic acid species and a non-significant decrease in faecal cholesterol excretion (Supplementary Fig. 1).

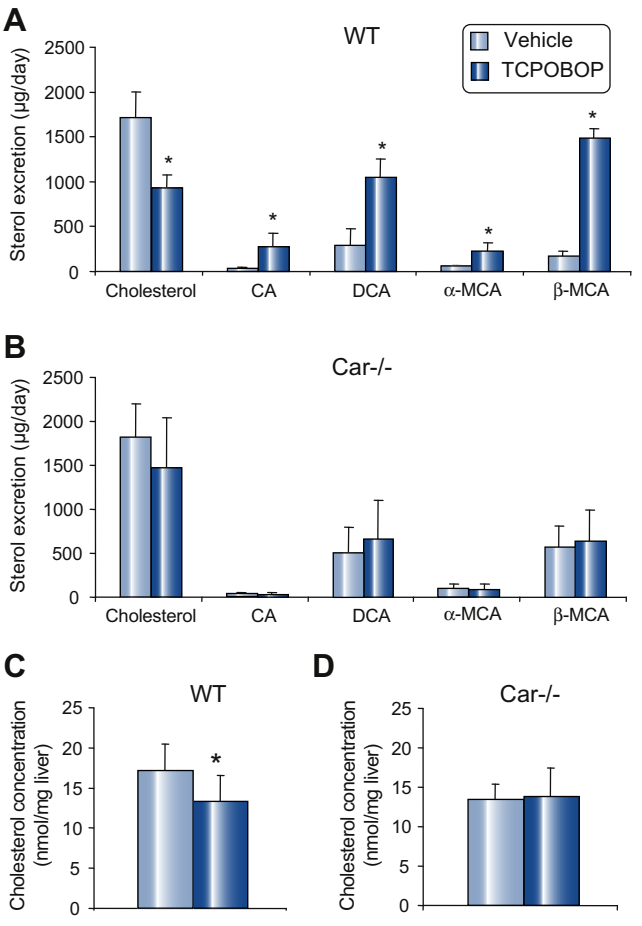

Fig. 1. Faecal sterol output and hepatic cholesterol concentration in WT and Car−/− mice treated or not with TCPOBOP. WT and Car−/− mice fed a western-type diet were treated weekly by i.p. injection of TCPOBOP or vehicle only for two weeks. (A and B) Faecal sterol excretion in WT and Car−/− mice. Faeces were collected over a 24 h period, five days after the last i.p. injection. Bile acid and neutral sterol contents of the faeces were determined by GC–MS. (C and D) Hepatic cholesterol concentration in WT and Car−/− mice. Hepatic cholesterol concentration was determined by an enzymatic method (∗ indicates significant differences from mice treated with the vehicle only, Student's *t*-test, $p < 0.05$, $n = 4$ in each group).