Steroids 125 (2017) 1–13

Contents lists available at ScienceDirect

Steroids

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

Review

Glucocorticoids and gut bacteria: "The GALF Hypothesis" in the metagenomic era

David J. Morris[a,*], Jason M. Ridlon[b,c,*]

[a] Department of Pathology and Laboratory Medicine, The Miriam Hospital, Warren Alpert Medical School of Brown University, Providence, RI, United States
[b] Department of Animal Sciences, Division of Nutritional Sciences, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, IL, United States
[c] Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, Richmond, VA, United States

ARTICLE INFO

Keywords:
Corticosterone
Cortisol
17α-Hydroxylase deficiency
Kidney 11β-hydroxysteroid dehydrogenase
Gut microbiota
Clostridium
Eggerthella
Glycyrrhetinic acid
Apparent mineralocorticoid excess
Essential hypertension
GALFs
21-Dehydroxylase
Steroid-17,20-desmolase

ABSTRACT

A new concept is emerging in biomedical sciences: the gut microbiota is a virtual 'organ' with endocrine function. Here, we explore the literature pertaining to the role of gut microbial metabolism of endogenous adrenocorticosteroids as a contributing factor in the etiology of essential hypertension. A body of literature demonstrates that bacterial products of glucocorticoid metabolism are absorbed into the portal circulation, and pass through the kidney before excretion into urine. Apparent mineralocorticoid excess (AME) syndrome patients were found to have congenital mutations resulting in non-functional renal 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2) and severe hypertension often lethal in childhood. 11β-HSD2 acts as a "guardian" enzyme protecting the mineralocorticoid receptor from excess cortisol, preventing sodium and water retention in the normotensive state. Licorice root, whose active ingredient, glycerrhetinic acid (GA), inhibits renal 11β-HSD2, and thereby causes hypertension in some individuals. Bacterially derived glucocorticoid metabolites may cause hypertension in some patients by a similar mechanism. Parallel observations in gut microbiology coupled with screening of endogenous steroids as inhibitors of 11β-HSD2 have implicated particular gut bacteria in essential hypertension through the production of glycerrhetinic acid-like factors (GALFs). A protective role of GALFs produced by gut bacteria in the etiology of colorectal cancer is also explored.

1. Introduction

Roughly 70 million Americans are afflicted with hypertension (1 of every 3 adults), a major contributor to the number one cause of death-cardiovascular disease [1,2]. Hypertension is viewed as a modifiable risk factor for cardiovascular disease, and the gut microbiome appears to play an important role in the etiology of hypertension. Indeed, several studies demonstrate structural and functional changes in the gut microbiome [3–5], causally associated with hypertension, as evidenced by phenotypic transfer of hypertension via stool from donor to recipient [6]. Studies have also shown that antibiotic treatment attenuates experimental hypertension although the mechanisms by which gut bacteria cause hypertension are unknown [7,8]. Similar phenotypic transfer of hypertension has been observed when stool from hypertensive human donors is transplanted into germ-free mice [9]. A major question remains what are the mechanisms by which gut microbiota affect host blood pressure? Steroid metabolism by gut bacteria has been understudied and a body of literature suggests a potential mechanism developed over the past fifteen to twenty years, known as "The GALF Hypothesis" which will be described in what follows.

Genetic defect in or inhibition of a renal isoform of the enzyme 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2) causes a severe form of hypertension [10]. Cortisol and aldosterone bind the mineralocorticoid receptor (MR) in the nephron with equal affinity, the occupation of which causes sodium and water retention, and thus elevated blood pressure. Circulating cortisol concentration is 100–1000 times greater than aldosterone. Consequently, the enzyme 11β-HSD2 acts as a "guardian", protecting MR from cortisol by converting it to cortisone, which has low affinity for MR [11,12]. Studies of gut bacterial steroid metabolism [13,14] coupled with studies on endogenous 11β-HSD2-inhibitors of adrenal origin [15–17] suggest a novel and unexplored mechanism by which gut bacterial metabolites increase blood pressure. The 11β-HSD1 isoform in vascular smooth muscle (VSM) and endothelium also plays a role in regulation of blood pressure (BP) [18]. 11β-HSD2 is present in vascular endothelial cells [18] and has also been suggested to be functional in MR regulatory processes in VSM [19].

* Corresponding authors at: Tel.: +1 401 741 3507 (D.J. Morris). Department of Animal Sciences, Division of Nutritional Sciences, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, IL, United States (J.M. Ridlon).
E-mail addresses: dmorris39@gmail.com (D.J. Morris), jmridlon@illinois.edu (J.M. Ridlon).

http://dx.doi.org/10.1016/j.steroids.2017.06.002
Received 14 May 2017; Accepted 12 June 2017
Available online 15 June 2017
0039-128X/

Here, we explore the available evidence linking gut microbial metabolism of host glucocorticoids with essential hypertension and make suggestions for future research.

2. The gut microbiome and hypertension

The gut microbiota are now recognized to play an important role in cardiovascular disease [3]. For instance, the production of trimethylamine-*N*-oxide (TMAO) in the liver, due to bacterial production of trimethylamine from dietary choline and l-carnitine, is a risk factor for atherosclerosis [20]. Fecal transplant of atherosclerosis-prone high TMAO-producing mice into *Ape*−/− low TMAO-producing mice resulted in increased atherosclerotic plaque formation implicating gut microbial metabolism of dietary components in atherosclerotic risk [21]. Studies have suggested a role for short chain fatty acids (SCFA) such as lactate in the regulation of blood pressure through binding to renal olfactory receptors and G-coupled protein receptor [22,23]. A recent, intriguing and unexpected observation was made in a rat model of salt-sensitive (S) vs. salt-resistance (R) hypertension that transfer of R cecal content to S rats resulted in significant, lifelong increase in systolic blood pressure [6]. Phenotypic transfer from hypertensive humans to mice was also recently reported, including detailed metagenomics and metabolomics analysis, although the mechanistic basis remains to be elucidated [9].

Phenotypic transfer of hypertension was recently reported in a rat model of obstructive sleep apnea by cecal transplant from hypertensive to normotensive rats [24]. The hypertensive state in this model was accompanied by significant increase in the family Coriobacteriaceae and decrease in the genus *Eubacterium*. However, the link between hypertension and gut microbiota remains obscure; the authors note lactate production by Coriobacteriaceae could affect renal olfactory receptors [24]. However, there are other attributes of particular strains of a species within the family Coriobacteriaceae that we will develop as an alternative hypothesis.

In a recent study, a notable observation was made suggesting that the transfer of hypertension was ablated by treatment with ampicillin and neomycin, suggesting a link between hypertension and the gut microbiota [24]. Other important studies by Honour et al. had previously demonstrated significant decrease in blood pressure during antibiotic treatment [4,7,25]. At this time, it needs to be pointed out that these links imply a correlation and not necessarily causation between gut microbes and hypertension in these animal models. The role of gut microbiota in experimental hypertension in rats induced by prolonged adrenal stimulation by adrenocorticotrophic hormone (ACTH) has been examined by measuring blood pressure response to corticosterone challenge in the presence or absence of oral antibiotic treatment [7]. Corticosterone treatment significantly increases systolic blood pressure, which is ablated by neomycin treatment [7]. Case reports have shown a link between reduction of resistant hypertension, that is, uncontrolled hypertension (blood pressure ≥140/90 mmHG on ≥3 antihypertensive drugs of different classes) and antibiotic treatment [26], possibly through immunomodulatory or other mechanisms. Similar studies with stroke prone spontaneously hypertensive rats (spSHR) also demonstrated a significant lowering of blood pressure following neomycin treatment [25].

It is probable that several mechanisms operate and contribute to the elevated BP under distinct subsets of essential hypertensive patients. Gut bacteria influence cardiovascular health, particularly through chronic low-grade inflammation, and gut microbiome composition is affected by physical activity, obesity, alcohol intake, and smoking, all of which are factors determining risk of hypertension [5,27].

3. Human glucocorticoids, cortisol and corticosterone

Corticosterone was the first steroid hormone isolated from adrenal gland extracts in 1937 [28–30], followed by deoxycorticosterone (DOC), the steroid precursor of corticosterone [31]. Early studies [32,33] showed that corticosterone caused a significant sodium retention and potassium secretion in Addisonian patients and possessed better life-sustaining properties than DOC in these same patients [33]. The identification of cortisone and the discovery that cortisol was the major glucocorticoid in humans was soon established [34,35].

Humans and many other higher mammalian species including monkeys, dog, sheep, cat, guinea pig, cattle, as well as certain fish; trout, sockeye salmon, herring, secrete both cortisol and corticosterone [36]. Corticosterone is synthesized at a rate approximately 1/10th that of cortisol [37,38]. Rodents lack adrenal 17α-hydroxylase (CYP17A) and only secrete corticosterone [37,38]. The adrenal secretion of both cortisol and corticosterone in humans, higher mammals, and other species suggests that the combination of two glucocorticoids may provide some evolutionary advantage(s) [39].

4. Metabolism of glucocorticoids by the host and isoforms of mammalian 11-hydroxysteroid dehydrogenase

Both cortisol and corticosterone can both be metabolized to their 5α- and 5β- Ring A- and C20α- and 20β-reduced derivatives by host enzymes [35,38,40]. In addition, the side-chain of cortisol can be cleaved by the enzyme 17α-hydroxylase/17,20-lyase (CYP17A) in peripheral tissues and possibly the adrenal to produce 11β-hydroxyandrostenedione or oxidized in the liver to corticoic acid metabolites [40–43]. Corticosterone in humans is metabolized in the liver principally to (allo)-3α,5α- but also to 3α,5β-tetrahydro(TH)-Ring A reduced metabolites before secretion into bile [35,37,39].

Two isoforms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2 and 1) are expressed in a variety of mineralocorticoid and glucocorticoid target tissues [44–51], where they interconvert active endogenous glucocorticoids (cortisol and corticosterone) to their respective 11-dehydro derivatives (cortisone and 11-dehydrocorticosterone) (Fig. 1). 11β-HSD1 functions as a bi-directional enzyme able to inactivate or re-activate endogenous glucocorticoids [44,46]. The reductase mode is favored in many tissues including liver, lung, etc. [48] but in other tissues, including vascular tissue, kidney proximal tubules, brain, and Leydig cells, 11β-HSD1 either operates as dehydrogenase or is truly bi-directional [49,52,53]. Recently this dehydrogenase activity has also been confirmed in vivo in human adipose tissue [54]. It appears most likely that the local redox-potential and local supply of substrates and co-factors regulate directionality of this isoform [50,51,55]. The 11-dehydro metabolites are generally considered to be biologically inert since they do not directly activate either glucocorticoid receptor (GR) or MR and induce a primary biologic effect. However, these metabolites have been proposed to serve a biological purpose; they can directly suppress the MR mediated actions of aldosterone; by an as yet, incompletely understood mechanism [56–58]. Glucocorticoids are known to amplify the vasoconstriction effects of both catecholamines and Angiotensin II in vascular tissue. These vasoconstriction effects are further enhanced by inhibitors of both 11β-HSD2 and 11β-HSD1 [18,49]. Thus, the GALF inhibitory substances (see below) generated by adrenal steroids including those from intestinal bacteria may well regulate the functional equilibrium of these enzymes in kidney and vascular tissue and hence lead to an increase in BP [49]. The realization by Edwards, Stewart, and Monder that 11β-HSD2 plays a major function to regulate the magnitude of MR mediated Na + retention in kidney represents a seminal discovery [11,12,47]. 11β-HSD1 had earlier been shown to be present in kidney, predominantly in the proximal tubules [11]. The confirmatory experiments demonstrating that 11β-HSD1 is present in kidney proximal tubules in a variety of species, including humans, and the observation that the 11β-HSD1 functions directionally as a dehydrogenase [52,53] requires in-depth investigation. Thus, if the dehydrogenase in this portion of the kidney tubule functions to inactivate cortisol, like 11β-HSD2 does in the distal tubules, 11β-HSD1 may also function as a “guardian” of