Mycophenolate

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Contributor: Juan Duarte

Microbiota is involved in the host blood pressure (BP) regulation. The immunosuppressive drug mofetil mycophenolate (MMF) ameliorates hypertension. The present study analyzed whether MMF improves dysbiosis in mineralocorticoid-induced hypertension. Male Wistar rats were assigned to three groups: untreated (CTR), deoxycorticosterone acetate (DOCA)-salt, and DOCA treated with MMF for 4 weeks. MMF treatment reduced systolic BP, improved endothelial dysfunction, and reduced oxidative stress and inflammation in aorta. A clear separation in the gut bacterial community between CTR and DOCA groups was found, whereas the cluster belonging to DOCA-MMF group was found to be intermixed. No changes were found at the phylum level among all experimental groups. MMF restored the elevation in lactate-producing bacteria found in DOCA-salt joined to an increase in the acetate-producing bacteria. MMF restored the percentage of anaerobic bacteria in the DOCA-salt group to values similar to control rats. The improvement of gut dysbiosis was associated with an enhanced colonic integrity and a decreased sympathetic drive in the gut. MMF inhibited neuroinflammation in the paraventricular nuclei in the hypothalamus.

Keywords: mycophenolate ; gut dysbiosis ; hypertension ; oxidative stress ; inflammation ; (deoxycorticosterone acetate) DOCA-salt model

1. Introduction

Systemic arterial hypertension is a complex, multifactorial, and multisystem disorder influenced by genetic and environmental factors, and is the most important modifiable risk factor that contributes significantly to worldwide cardiovascular morbidity and mortality. A link between gut microbiota to hypertension in both animal models and human hypertension has been described. Recently, an imbalance in the gut microbiota composition relative to the healthy state, termed dysbiosis, has been associated with hypertension [1][2][3][4]. The characteristics of dysbiosis are different between systemic renin–angiotensin system (RAS)-dependent hypertension, such as in spontaneous hypertensive rats (SHR) [1][3][5][6][7], and systemic RAS-independent forms, such as hypertension induced by mineralocorticoid receptor activation [8][9]. Studies using fecal microbiota transplantation procedures have demonstrated that gut microbiota from hypertensive animals and human increased blood pressure (BP), showing a cause–effect relationship [3][10][11], albeit the mechanisms involved in BP regulation by the microbiota have not been fully elucidated.

Emerging evidence indicates that immune system dysfunction is an important factor in the pathogenesis of hypertension [12]. In fact, T cell activation is involved in the development of hypertension induced by angiotensin II infusion and by deoxycorticosterone acetate (DOCA)-salt [13]. The microbiome plays a critical role in the induction, maturation, and maintenance of the host immune system. Interestingly, inhibition of T cell activation and helper T (Th)17 differentiation reduced the hypertensive effect induced by dysbiotic microbiota from SHR, showing that immune system dysregulation induced by the gut microbiota may be, at least partially, responsible for the development of hypertension [3]. Interestingly, the improvement of gut dysbiosis induced by probiotic bacteria [7][9][14], dietary fiber [8], or by drug treatment [6][15], in several experimental models of hypertension, was involved in their antihypertensive effects. However, the antihypertensive drug hydralazine was unable to improve gut dysbiosis in SHR despite intensive BP reduction, showing that gut microbiota composition was not adapted to the host health status of normotension [6]. Recently, Raizada's group has linked hypothalamic neuroinflammation and increased sympathetic drive with changes in gut physiology and microbiota associated with angiotensin II-induced hypertension [16]. This suggests a role for a dysfunctional autonomic nervous system in gut dysbiosis.

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA), an inhibitor of inosine 5'-monophosphate dehydrogenase. This drug depletes proliferating B and T lymphocytes and macrophages due to its ability to rate-limit enzymes in de novo synthesis of guanosine nucleotides. MMF was able to prevent the development of hypertension in DOCA-salt due to pharmacological inhibition of B and T cell proliferation [17][18]. Recently, it has been described that MMF influences the gut microbiome in renal transplant patients [19][20][21][22] and rodents [23]. Renal transplant recipients suffer from dysbiosis, characterized by lower diversity and loss of butyrate-producing bacteria, more than one year post-

transplantation, and the use of MMF correlates to a lower diversity [21]. In addition, MMF exposure promoted expansion of the phylum Proteobacteria. This increase was accompanied by gene enrichment for multiple bacterial enzymes involved in the biosynthesis of lipopolysaccharide (LPS) and increased plasma levels of LPS [19]. Thus, lower diversity in gut microbiota and higher plasma LPS levels induced by MMF could be harmful under hypertensive conditions [1][3][7]. By contrast, the reduction in blood pressure and gut sympathetic tone induced by MMF could improve dysbiosis linked to hypertension. However, it is unknown as to whether the modulation of the immune system by MMF improves the remodeling of gut microbiota under mineralocorticoid-induced hypertensive conditions. Thus, the aim of this study was to analyze the effects of MMF in the gut microbiota in this model of hypertension, focusing on the involvement of the sympathetic nervous system.

2. Discussion

Abundant evidence has demonstrated the association between gut dysbiosis, the immune system, and hypertension [1][3][4][11][24]. Our results are consistent with data previously described, with MMF being able to prevent BP increase [17][18] and improve aortic endothelial dysfunction, increasing NO availability in DOCA-salt animals [18]. Furthermore, we found that the MMF treatment induced a modulation in the aortic immune cell infiltration, inducing a reduction in the pro-inflammatory and pro-oxidative cytokine profile.

Several studies have described the ability of immunomodulatory drugs to modulate the gut microbiota, inducing dysbiosis [25] or improving the dysbiotic condition found in several pathologies [26]. Last year, the way in which gut dysbiosis is displayed in DOCA-salt hypertension was reported [8][9]. Our results are in agreement with the key known characteristics of gut microbiota described in DOCA-salt animals [8][9], such as a reduced evenness, no change in the F/B ratio, and a higher proportion of lactate-producing bacteria. MMF treatment induced a remodeling in the gut microbiota, normalizing the proportion of bacteria belonging to Firmicutes and Bacteroidetes and reducing lactate-producing bacteria. In addition, MMF increased acetate-producing bacteria, which could contribute to reduce BP. In fact, the increase in acetate-producing bacteria induced by high-fiber diet, *Bifidobacterium breve* consumption, or acetate supplementation were associated with decreased BP, improvement of vascular endothelial and cardiac dysfunction, and attenuation of cardiac and renal fibrosis in DOCA-salt animals [8][9].

BP-lowering effects of MMF have been associated with decreased circulating and renal T cells in SHR and DOCA-salt rats, although MMF reduced both T cell subtypes, Th17 and Tregs [17][18][27] However, we found increased Tregs and IL-10 (the main cytokine produced by Treg) in both MLNs and aortas of MMF-treated rats, which could contribute to their antihypertensive effects, since IL-10 released by Tregs improves endothelial function and reduces BP in hypertensive mice [28]. Several authors have described the ability of certain SCFAs, such as acetate and butyrate, to modulate the immune system. Concretely, acetate is able to induce an elevation in Treg populations [9][29]. We found an expansion in acetate-producing bacteria induced by MMF treatment in DOCA-salt rats, which could be involved in the higher numbers of Treg and IL-10 found in MLN and aorta from the DOCA-MMF group. Otherwise, in DOCA-salt rats, we found an elevation in the genus *Sutterella*, which has been described to elevate Th17 populations [30]. MMF reduced *Sutterrella* contents in feces from DOCA-salt rats, possibly leading to lower Th17. This effect in the microbiota could increase the direct inhibitory effect of MPA, the active form of MMF, inhibiting IL-17 expression [31]. In addition, the increased abundance of *Lactobacillus* spp. found in DOCA samples was also normalized by the MMF treatment, similarly to acetate consumption [9]. This is an important beneficial effect because *Lactobacillus* spp. has been shown to elevate certain pro-inflammatory cytokines such as IL-6, tumor necrosis factor (TNF)α, or interferon (INF)γ in enterocytes [32][33].

Multiple possibilities wherein MMF might elicit changes in gut microbiota were found. It has been repeatedly shown how a change in the host health status is accompanied by changes in the composition of gut microbiota. Therefore, microbiota could be adapted to BP reduction, shifting to a microbiota composition similar to normotensive rats. However, we previously demonstrated that hydralazine, which normalized BP in SHR, was unable to improve dysbiosis [6], ruling out the hypothesis that gut microbiota are adapted to normotensive conditions. Changes in gut microbiota composition have been associated with gut integrity [2][6]. The mammalian digestive tract epithelial cells create a tight barrier in the gut, contributing to the hypoxic environment of the lumen. Damage to this barrier makes the environment less hypoxic, conducive to aerobic bacterial growth [34][35]. We found a significant reduction in the mRNA expression of tight junction protein ZO-1 and mucins in colon from DOCA-salt rats, suggesting reduced colonic integrity and increased gut permeability in hypertensive rats. The possible impairment of gut barrier function was supported by the translocation of endotoxin from the intestinal lumen to the bloodstream, leading to higher LPS plasma levels in the DOCA-salt group. MMF treatment increased colonic ZO-1 expression and normalized MUC-2 and MUC-3 mRNA levels, suggesting improvement of gut barrier function. In fact, MMF inhibited the LPS translocation to the systemic circulation. In addition, intestines of angiotensin II-hypertensive mice and SHR were significantly less hypoxic and with increased aerobic bacteria in feces,

due to a reduction in the epithelium barrier integrity [2][6]. We also found decreased abundance of anaerobic bacteria in feces from DOCA-salt rats, which was associated with a loss of gut integrity. DOCA-salt rats treated with MMF showed increased colonic integrity and a proportion of strict anaerobic bacteria similar to control groups. These data reinforce the key role of gut integrity in the composition of intestinal microbiota. In addition, intestinal epithelial cells and Paneth cells secrete antimicrobial peptides, such as defensins, which selectively kill Gram-positive bacteria [36][37][38][39]. Components of the microbiota, such as LPS, are recognized by Toll-like receptors expressed by these intestinal cells and trigger production and secretion of these defensins. We found changes in the expression levels of defensins in colonic samples from the DOCA group in comparison with control group, which might also be involved in changes in microbiota found in hypertensive rats. MMF restored defensin expression to become similar to that found in normotensive rats. Overall, MMF treatment, by increasing acetate-producing bacteria and possibly acetate content in feces, might increase gut barrier function, reducing endotoxemia and improving Th17/Treg balance in MLNs, reducing Th17 infiltration in vascular tissues, which participate in reducing BP. However, whether the remodeling induced by MMF in gut microbiota contributes to lower BP in DOCA-salt rats requires further investigation using fecal microbiota transplantation from the donor DOCA-MMF group to recipient hypertensive DOCA-salt rats.

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