Local corticosterone production and angiotensin-Ⅰ converting enzyme shedding in a mouse model of intestinal inflammation

Hanne Salmenkari, Tomi Issakainen, Heikki Vapaatalo, Riitta Korpela

Hanne Salmenkari, Tomi Issakainen, Heikki Vapaatalo, Riitta Korpela, Faculty of Medicine, Pharmacology, University of Helsinki, 00290 Helsinki, Finland

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Correspondence to: Heikki Vapaatalo, Professor Emeritus, Faculty of Medicine, Pharmacology, University of Helsinki, Haartmaninkatu 8, 00290 Helsinki, Finland.

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Abstract

AIM: To investigate local corticosterone production and angiotensin-Ⅰ converting enzyme (ACE) protein expression and their interaction in healthy and inflamed intestine.

METHODS: Acute intestinal inflammation was induced to six weeks old male Balb/c mice by administration of either 3% or 5% dextran sodium sulfate (DSS) in drinking water for 7 d (n = 12 in each group). Healthy controls (n = 12) were given tap water. Corticosterone production and ACE protein shedding were measured from ex vivo incubates of the small and large intestine using EIA and ELISA, respectively. Morphological changes of the intestinal wall were assessed in hematoxylin-eosin stained tissue preparations of jejunum and distal colon. Effects of angiotensin II, captopril and metyrapone on corticosterone production was assessed by incubating pieces of small intestine of healthy mice in the presence of 0.1, 1 or 10 µmol/L angiotensin II, 1, 10 or 100 µmol/L captopril or 1, 10 or 100 µmol/L metyrapone solutions and measuring corticosterone released to the incubation buffer after 90 min (n = 5 in each group).
RESULTS: Both concentrations of DSS induced inflammation and morphological changes in large intestines but not in small intestines. Changes were observed as distortions of the crypt structure, mucosal erosion, immune cell infiltration to the mucosa and submucosal edema. *Ex vivo* corticosterone production (2.9 ± 1.0 ng/mL vs 2.0 ± 0.8 ng/mL, *P* = 0.034) and ACE shedding (269.2 ± 97.1 ng/mL vs 175.7 ± 52.2 ng/mL, *P* = 0.016) were increased in small intestines in 3% DSS group compared to the controls. In large intestine, corticosterone production was increased compared to the controls in both 3% DSS (229 ± 81 pg/mL vs 158 ± 30 pg/mL, *P* = 0.017) and 5% DSS groups (366 ± 163 pg/mL vs 158 ± 30 pg/mL, *P* = 0.002). Large intestine ACE shedding was increased in 5% DSS group (41.5 ± 9.0 ng/mL vs 20.9 ± 5.2 ng/mL, *P* = 0.034). Angiotensin II treatment augmented corticosterone production in small intestine at concentration of 10 μmol/L (0.97 ± 0.21 ng/mg protein vs 0.40 ± 0.09 ng/mg protein, *P* = 0.036).

CONCLUSION: Intestinal ACE shedding is increased by DSS-induced intestinal inflammation and parallels local corticosterone production. ACE product angiotensin II stimulates corticosterone formation in healthy intestine.

Key words: Dextran sodium sulfate; Inflammation; Angiotensin-I converting enzyme; Local corticosterone; Intestine

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Core tip: Soluble and tissue levels of angiotensin-I converting enzyme (ACE) along with corticosterone production were examined in a dextran sulfate mouse model of intestinal inflammation. Intestine is a site of ACE shedding, which is increased by inflammation. ACE and corticosterone are increased in intestinal incubations of morphologically disrupted and intact parts of the intestine. ACE product Ang II stimulates corticosterone production in small intestine. The results suggest that intestinal Renin-Angiotensin system and glucocorticoids might be counter-regulatory systems in regulation of inflammatory processes in the intestine.


INTRODUCTION

Renin-angiotensin system (RAS) is best known as a regulator of systemic blood pressure. In addition, classic and alternative RAS regulate inflammatory processes in the vasculature[1]. Several components of RAS have been localized in various parts of the gastrointestinal tract but their function is not completely clear[2]. Angiotensin converting enzymes (ACE, ACE2) have been found throughout the human intestine[3-5]. The two types of angiotensin receptors (AT1R, AT2R) have also been detected in rat and human intestine suggesting that ACE aminopeptidases are not only food metabolizing enzymes but also have regulatory functions[2-4-7].

Another recent observation is the formation of adrenocortical glucocorticoid (GC) hormone, corticosterone, in the gut, where the regulation of synthesis is different than in adrenals[8-10]. Intestinal epithelium produces corticosterone to regulate inflammation by the action of tumor necrosis factor (TNF)-α[11]. In kidney and heart, angiotensin II (Ang II) induces TNF-α production[12,13]. Therefore, we hypothesized that these two systems, pro-inflammatory RAS and anti-inflammatory GCs, could play a counter-regulatory role in inflammatory processes of the intestine.

ACE is the central enzyme of classic RAS. ACE is an aminopeptidase, which cleaves two or three C-terminal amino acids from several peptides. The most important substrate for ACE is angiotensin I (Ang I) which is cleaved into pro-inflammatory Ang II. ACE is a membrane-bound enzyme with a short cytosolic C-terminal tail[14]. The extracellular part consists of N-terminal and C-terminal domains which both possess a catalytic site[15]. ACE extracellular domains can be cleaved and released to the circulation by one or more so called ACE sheddase enzymes[15-17]. One of those enzymes is a metalloprotease ADAM9[18]. An analogous shedding mechanism by ADAM17 has been described for ACE2[15,20]. The role of ACE shedding is unclear but it is thought that ACE shedding might be a way to regulate local ACE activity or substrate specificity[15]. Furthermore, ACE shedding has been reported in lung during ischemia/reperfusion and in pulmonary endothelial cells in septic conditions and during LPS treatment *in vitro*[18,21,22]. Here, we report of ACE shedding outside vasculature from the intestinal tissue in response to DSS-induced inflammation.

Dextran sulfate (DSS)-model of colitis induces inflammation, mucosal erosion and bleeding in mouse colon. There have been several reports of DSS inducing mild inflammatory changes in small intestine histology and biochemical markers up to jejunum[23-25].

The aim of the study was to investigate intestinal corticosterone production and ACE protein expression and their interaction in healthy and inflamed intestine.

MATERIALS AND METHODS

The study was conducted in the Institute of Bio-
Inflammatory cells and submucosal edema were observed in the large intestine preparations, marked disruption of crypt structure, mononuclear cell infiltration, submucosal edema and mucosal erosion were observed in both 3% and 5% DSS groups but were not present in the control group (Figure 1). Histological changes were similar in both colitis groups. No pathological changes were observed in the small intestines in any group.

**Histological analyses**  
Formalin-fixed paraffin embedded tissues were sliced and stained with hematoxylin and eosin stain. Crypt and villus structure, erosion of mucous membrane, inflammatory cells and submucosal edema were visually evaluated and compared between treatments.

**Corticosterone production**  
Corticosterone production in the ex vivo incubation of the small intestine samples was increased in 3% DSS group compared to control group at 90 min (2.9 ± 1.0 ng/mL vs 2.0 ± 0.8 ng/mL, \( P = 0.034 \)) (Figure 2A). However, corticosterone levels did not differ significantly from control in 5% DSS-treated mice's small intestines (2.5 ± 0.9 ng/mL vs 2.0 ± 1.0 ng/mL, \( P = 0.16 \)). In large intestine, DSS treatment stimulated corticosterone production in both 3% DSS groups.
compared to control (41.5 ± 23.8 ng/mL vs 20.9 ± 13.7 ng/mL, \( P = 0.034 \)) but not in 3% DSS group. In tissue lysates, ACE protein concentrations were similar in all groups (Figure 2E and F).

Corticosterone and ACE correlations
Corticosterone and ACE concentrations correlated with each other in both small intestine (Pearson correlation 0.435, 95%CI: 0.169-0.642, \( P = 0.001 \)) and large intestine (Pearson correlation 0.315, 95%CI 0.033-0.550, \( P = 0.015 \)) (Figure 3). Similarly, there was a correlation between small and large intestine corticosterone production (Pearson correlation, 0.534, 0.295 to 0.711, \( P < 0.001 \)) but not in ACE shedding (Pearson correlation 0.027, \( P = 0.429 \)).

ACE shedding
ACE protein was present in the supernatants of mouse ex vivo intestinal incubations (Figure 2C and D). ACE concentration was increased in 3% DSS group small intestine supernatants at 90 min compared to control (269.2 ± 90.9 ng/mL vs 175.7 ± 48.8 ng/mL, \( P = 0.016 \)). In large intestine incubates, ACE concentrations were increased in 5% DSS group compared to control (41.5 ± 23.8 ng/mL vs 20.9 ± 13.7 ng/mL, \( P = 0.034 \)) but not in 3% DSS group. In tissue lysates, ACE protein concentrations were similar in all groups (Figure 2E and F).
Effects of angiotensin II, captopril and metyrapone on corticosterone production in healthy tissue

Angiotensin II, a stimulator of adrenal corticosterone synthesis, also increased corticosterone production in small intestines at 10 µmol/L concentration (0.97 ± 0.04 ng/mg protein vs 0.40 ± 0.02 ng/mg protein, P = 0.036), which was the highest concentration tested (Figure 4A). Captopril, an inhibitor of ACE, had no effect on corticosterone production (Figure 4B). Metyrapone, an inhibitor of corticosterone synthesis, unexpectedly increased corticosterone production at the smallest concentration tested, 1 µmol/L (0.95 ± 0.41 ng/mg protein vs 0.40 ± 0.02 ng/mg protein, P = 0.040), but had no effect at higher concentrations (Figure 4C).
DISCUSSION

Inflammatory bowel diseases (IBDs) are severe clinical ailments which manifest by diarrhea, intestinal bleeding and inflammation. Two major forms of IBD are ulcerative colitis (UC) and Crohn’s disease (CD). In UC, the inflammation is restricted to the colonic mucosa, whereas in CD the inflammation can sporadically affect any part of the intestinal tract and reach through all tissue layers. The causes of IBD are not fully understood but they are thought to be multifactorial and involve hereditary, immunological and environmental factors.

DSS is widely used to induce inflammation of the large intestine as an animal model of IBD\(^{[26]}\). However, it has been reported to induce inflammation in the small intestine as well, mainly in the ileum. In the present study, DSS treatment caused extensive histological damage in the mucosal layer of mouse colon but did not cause any clear histological damage in the jejunum. No appreciable changes were observed in muscularis mucosae, bearing more resemblance to ulcerative colitis than Crohn’s disease.

In the present study, we tested, as far as we know,
Intestinal corticosterone production and ACE shedding

for the first time the possible association between glucocorticoids and renin-angiotensin system in the gastrointestinal tract. These two systems interact in the adrenal cortex.

Here, we report of inflammation-induced ACE protein shedding from mouse intestine in an ex vivo incubation. ACE shedding from vascular endothelium has been demonstrated earlier by others. Previously, ACE activity has been measured from feces and considered as a sign of cell damage. The current study did not assess whether ACE shedding occurs from the epithelial cells, immune cells or the vascular endothelium. However, due to the handling of the samples and recent reports of an ACE sheddase, it is likely the measured protein is in soluble form rather than membrane-bound. Furthermore, in severe colitis, we have observed angiotensinogen expression coinciding with ACE shedding (unpublished data).

Intestinal corticosterone production is increased by inflammation. In line with previous studies, corticosterone production was increased in large intestines of DSS-treated mice. Furthermore, despite no histological changes were observed in small intestines of DSS-treated mice, corticosterone production and ACE shedding were increased in jejuna of DSS-treated mice without marked differences in the tissue ACE levels. This might be due to milder, non-detectable, inflammation of the small intestine. Since both of these processes, corticosterone production and ACE shedding, were increased by inflammation, we hypothesized that they might interact in the intestine. Indeed, corticosterone production was increased in small intestine tissue incubation by Ang II. However, inhibition of ACE by captopril in vitro did not affect the basal corticosterone production. Further studies will show, whether captopril treatment in vivo has an effect on corticosterone production in inflamed intestine. Interestingly, the in vivo glucocorticoid synthesis inhibitor metyrapone did not inhibit corticosterone synthesis in this in vitro experimental setup but rather increased it at low concentration.

The present study shows that the DSS colitis model can be used to study local angiotensin system in intestinal inflammation. As conclusion, we demonstrate ACE shedding from mouse intestine induced by inflammation. Pro-inflammatory ACE and anti-inflammatory glucocorticoids appeared in incubation buffer even in the small intestine, far from the site of structural damage, further supporting reports of small intestinal manifestations of the DSS model. The results of this study suggest that intestinal RAS and glucocorticoids might be counter-regulatory systems in regulation of inflammatory processes in the intestine.

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