Scandinavian Journal of Gastroenterology

ISSN: 0036-5521 (Print) 1502-7708 (Online) Journal homepage: http://www.tandfonline.com/loi/igas20

The renin–angiotensin system in Barrett's esophagus

Svein Olav Bratlie, Anders Edebo, Anna Casselbrant, Herbert F. Helander & Lars Fändriks

To cite this article: Svein Olav Bratlie, Anders Edebo, Anna Casselbrant, Herbert F. Helander & Lars Fändriks (2016): The renin–angiotensin system in Barrett's esophagus, Scandinavian Journal of Gastroenterology

To link to this article: http://dx.doi.org/10.1080/00365521.2016.1174881

Published online: 13 May 2016.

SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, 2016
http://dx.doi.org/10.1080/00365521.2016.1174881

Taylor & Francis
Taylor & Francis Group

The renin–angiotensin system in Barrett's esophagus

Svein Olav Bratlie*, Anders Edebo*, Anna Casselbrant, Herbert F. Helander and Lars Fändriks

Department of Gastrosurgical Research and Education, Institute of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

ABSTRACT

Objective: Barrett's esophagus (BE) is a risk factor for esophageal adenocarcinoma. In addition to its classical endocrine character known for hemodynamic regulation, the renin–angiotensin system (RAS) can be associated with inflammation, wound healing, and cancer. The aim of this study was to explore a potential expression of the RAS in BE, with or without the presence of dysplasia.

Material and methods: Biopsy material was prepared for western blotting and immunohistochemistry. Non-BE patients (controls) were compared with BE patients regarding RAS in the squamous epithelium. In the columnar BE mucosa, RAS expression was studied in patients with and without dysplasia. Key components of the 'classical' RAS were assessed: the angiotensin-converting enzyme (ACE) and the angiotensin II subtype 1 and 2 receptors (AT1R and AT2R).

Results: The presence of RAS factors was confirmed in the esophageal mucosa of both control and BE patients. ACE protein expression was 48% lower ($p = 0.001$) whereas AT1R was 45% higher ($p = 0.039$) in the squamous epithelium of BE patients compared to epithelia from non-BE controls. In the metaplastic intestinal-like epithelium, AT1R expression was 37% higher in BE patients with confirmed dysplasia than in patients without dysplasia ($p = 0.009$). Immunohistochemistry showed an altered distribution of RAS proteins in BE patients with dysplasia.

Conclusions: The differential RAS expression observed may prove to be useful as a biomarker or a pharmaceutical target.

ARTICLE HISTORY
Received 8 March 2016
Revised 31 March 2016
Accepted 2 April 2016
Published online 10 May 2016

KEYWORDS
Barrett's esophagus; biomarkers; endoscopy; esophageal adenocarcinoma; renin–angiotensin receptor

Introduction

Barrett's esophagus (BE) is strongly associated with an increased risk of development of esophageal adenocarcinoma (EAC). The prevalence of EAC has increased markedly during the last decades.[1–3] The neoplastic progression towards EAC is believed to develop through a series of dysplastic transformations. A large number of pathogenic factors have been claimed to be involved, making the picture far from clear.[4] Surveillance of BE for early detection of pro-neoplastic lesions relies solely on endoscopy with tissue sampling and histopathological evaluation. Less invasive surveillance methods are urgently needed, such as biomarkers indicating neoplastic progression.

The renin–angiotensin system (RAS) has for decades been known to be involved in fluid and electrolyte homeostasis, and in hemodynamic regulation. There is growing evidence that this endocrine regulatory system also has a tissue-based element in most organs, e.g., the brain,[5] the kidney,[6] the adrenals,[7] the pancreas,[8] the liver,[9] and the colon.[10] Furthermore, the RAS is apparently involved in several pathology-related conditions such as inflammation and wound healing.[11,12] Interestingly, the RAS has also been implicated in carcinogenesis.[13] 'Classical' regulatory actions by RAS are mediated by the octapeptide angiotensin II (AngII), which is formed by the angiotensin-converting enzyme (ACE). The cell-surface-bound angiotensin II receptor type 1 (AT1R) raises blood pressure by inducing vasoconstriction and renal sodium retention. The angiotensin II type 2 receptor (AT2R), normally has a restricted distribution but can be induced in various pathological conditions and mediates anti-inflammatory functions and tissue restitution. Binding of AngII to either AT1R or AT2R is thought to have different effects (synergistic or opposing) and the distribution of surface receptors defines the response to AngII (e.g., vasoconstriction or vasodilatation).[14,15] The role of the RAS in gastrointestinal physiology and disease has so far been poorly explored.[16] In a British epidemiological study, Sjöberg et al. (2007) noted a lower prevalence of EAC in patients treated with RAS-interfering antihypertensive drugs such as AT1R blockers and ACE inhibitors.[17] Results from our laboratory have indicated the existence of a local RAS in the musculature of the esophageal wall [18] and in the squamous mucosa.[19] This was further explored by Björkman et al, who found that some RAS components are significantly different in patients with erosive reflux disease from those in healthy volunteers.[20] In a *post hoc* analysis on patients treated with proton pump inhibitors for reflux esophagitis, Miwa et al. discovered enhanced recovery when AT1R blockers were added.[21] RAS components have recently been reported to be involved in various malignant states, e.g., in pancreatic cancer.[22] Based on these data we hypothesised that the RAS is involved in the progression

CONTACT Dr Svein Olav Bratlie svein.olav.bratlie@vgregion.se Department of Gastrosurgical Research and Education, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden
*These authors contributed equally.

from benign Barrett's metaplasia to the precancerogenic dysplastic state.

The present study was undertaken to test this hypothesis by exploring the expression of the RAS factors in BE, with or without the presence of dysplasia. Another aim was to compare RAS expression in the squamous epithelium of BE patients with normal esophageal mucosa of control patients. We concentrated on the 'classical' RAS mediator AngII by assessing the possible presence of its receptors AT1R and AT2R and of its principal synthesising enzyme ACE.

Methods

The study was approved by the ethical committee of Gothenburg University and by the Regional Ethical Review Board in Gothenburg, and was performed in accordance with the Declaration of Helsinki. All study participants were informed verbally and in writing, and signed a consent form.

Study subjects

BE patients

Patients who had been referred to our unit for surveillance endoscopy were asked to participate in this study and 79 accepted. Patients on medication that would interfere with the RAS (i.e., AT1R blockers and ACE inhibitors) and those with a previous record of surgery in the upper gastrointestinal tract were excluded. In total 42 BE patients scheduled for endoscopy were included of which 26 were non-dysplasia patients with a mean age of 61 y (min–max: 44–74 y; nine females) and 16 were diagnosed with low-grade dysplasia; mean age 66 y (min–max: 58–86 y; one female). Apart from the already scheduled Barrett-surveillance biopsies, another 4–6 study biopsies were taken for research purposes. In order to optimize the immunohistochemical localisation of RAS components in dysplastic mucosae we enrolled eight patients with a mean of age 65 y (min–max: 42–76 y; two females) who were scheduled for esophagectomy due to diffusely spread HGD in the Barrett mucosa that was not suitable for endoscopic resection. In these patients, mucosal biopsies were obtained peroperatively.

Control patients

Twelve consecutive patients referred to the endoscopy department for diagnostic evaluation of disorders not related to esophageal disease (e.g., anemia or suspected ulcer disease) were asked to participate. Patients with RAS-interfering medication were excluded. These patients completed the Carlsson-Dent questionnaire validated for the diagnosis of gastro-esophageal reflux disease based on symptoms.[23] Only esophageal mucosal biopsies from patients with low scores, i.e., no reflux disease, and without endoscopic signs of gastro-esophageal reflux were included ($n = 7$) and the mean age of this group was 67 y (min–max: 48–83; two females).

Endoscopy and biopsy procedure

Mucosal specimens were collected with the participants placed in the left lateral position. Endoscopy was carried out using a high-definition magnifying endoscope (Fujinon EG485ZH or EG495ZH; Fujifilm, Tokyo, Japan). In the non-BE control patients, the squamo-columnar junction (SCJ) was thoroughly investigated to rule out the presence of asymptomatic erosive reflux disease. Biopsies were taken from squamous epithelium at the 3 o'clock position immediately above the SCJ. In BE-patients biopsies were taken in the 3 o'clock position immediately above the gastro-esophageal junction, demarcated by the proximal limit of the longitudinal gastric folds. Samples of squamous mucosa were also taken at the 3 o'clock position but immediately above the orally displaced SCJ. In each location, paired or triple biopsies were taken in close proximity to each other and were handled according to the methods described below.

Histopathology

Biopsies were fixed in buffered 4% formalin, dehydrated, and embedded in paraffin. For evaluation of general histology, 3-μm sections were mounted on slides and stained with haematoxylin and eosin. Each biopsy was examined and categorized according to the Vienna classification,[24] performed in an unblinded routine manner by two experienced histopathologists at the Department of Pathology, Sahlgrenska University Hospital.

Western blot analysis

Biopsy specimens were snap-frozen in liquid nitrogen and kept frozen for later western blot analysis of ACE, AT1R, and AT2R expression. Briefly, the frozen specimens were sonicated in PE buffer (10 mM potassium phosphate buffer, pH 6.8, and 1 mM EDTA) containing 10 mM 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulphonate (CHAPS; Boehringer Mannheim, Mannheim, Germany) and protease inhibitor cocktail tablet Complete (Roche). The homogenate was then centrifuged (10,000*g* for 10 min at 4 °C) and the supernatant was analysed for protein content according to the method of Bradford.[25] Samples were diluted in SDS buffer and heated at 70 °C for 10 min before they were loaded on a NuPage 10% Bis–Tris gel, and electrophoresis was run using MOPS buffer (Invitrogen). One lane of each gel was loaded with pre-stained molecular weight standards (SeeBlue; NOVEX, San Diego, CA). KNRK (for AT2R), PC-12 (for AT1R), and kidney extract (for ACE) whole-cell lysates as appropriate served as positive controls (Santa Cruz Biotechnology, CA). After the electrophoresis, the proteins were transferred to a polyvinyl difluoride membrane (Amersham, Buckinghamshire, UK), which was incubated with antibodies to ACE, AT1R, and AT2R, respectively. An alkaline phosphatase-conjugated donkey anti-goat or goat anti-rabbit IgG antibody (Santa Cruz) and CDP-Star (Tropix, Bedford, MA) was used as a substrate to identify immunoreactive proteins by means of chemiluminescence. Images were captured on a Chemidox XRS cooled CCD camera and analysed with Quantity One software (Bio-Rad Laboratories, Hercules, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; IMG-5143A, Imgenex; BioSite, San Diego, CA) was used as control for equal loading and for each sample tested the optical

density of primary antibody/GAPDH corresponds to the result (Figure 1).

Immunohistochemistry

Sections for immunohistochemistry were de-paraffinised and boiled for 15 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. After blockade of endogenous peroxidase activity, slides were pre-incubated with serum block followed by incubation with primary antibodies to either ACE, AT1R, or AT2R for 1 h at room temperature, at dilutions of 1:100. The primary antibodies were raised in goat, rabbit, and goat, respectively (Santa Cruz). Control sections were incubated with normal goat IgG or rabbit IgG at 0.4 μg/μL instead of the primary antibody. After being washed, the slides were incubated with biotinylated secondary antibody and the complex was detected using horseradish peroxidase-streptavidin. The colour was developed using 3,3′-diaminobenzidine.

Statistics

All statistical analyses were performed with SPSS statistical software version 20.0 (IBM SPSS, Chicago, IL). The Shapiro–Wilk's test indicated that the data were not normally distributed and the non-parametric Kruskal–Wallis test and the Mann–Whitney *U* test were used, with significance being assumed at $p \leq 0.05$.

Results

RAS protein expression

Squamous epithelium

The expression of ACE protein was significantly lower in the squamous epithelium of BE-ND patients than in samples from control subjects ($p = 0.001$). A similar tendency was noted regarding BE-LGD patients, but this difference did not reach statistical significance ($p = 0.067$) (Figure 2(a)). The expression of AT1R was significantly higher in BE patients, both in the BE-ND group ($p = 0.039$) and the BE-LGD group ($p = 0.018$), than in control subjects (Figure 2(b)). AT2R protein expression in squamous epithelium did not differ between control subjects and BE patients, regardless of whether or not there was dysplasia (Figure 2(c)).

Barrett mucosa

AT1R levels in BE were found to be significantly higher in patients with dysplasia than in patients with no dysplasia ($p = 0.009$) (Figure 3(b)). The levels of ACE and AT2R did not differ significantly between the BE-ND patients and the BE-LGD patients (Figure 3(a,c)).

Localisation of RAS

The intraepithelial distribution of proteins AT1R, AT2R, and ACE was assessed by immunohistochemistry using specimens from BE-ND patients ($n = 6$) and BE-HGD patients ($n = 8$). The biopsies generally showed weak staining by the anti-AT1R antibody in the lamina propria, with blood vessel walls that were mostly unstained. In columnar cell epithelium from BE-ND patients, both luminal and glandular crypts were stained (Figure 4(a)), whereas in patients diagnosed with BE-HGD the staining of AT1R was generally absent in glandular crypts and comparatively weak in the luminal surface cells (Figure 5(a)). Epithelial AT2R staining was observed in BE-ND specimens, but was absent in BE-HGD specimens. In contrast, vascular structures in the lamina propria were distinctly stained for AT2R in BE-HGD patient samples but not in the BE-ND patient samples (Figures 4(b) and 5(b)).

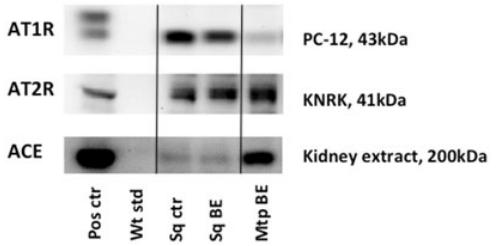

Figure 1. Western blot gel analysis of proteins in patient samples. The first lane was loaded with positive controls (Pos ctr) for angiotensin II receptor types 1 and 2 (PC-12 for AT1R and KNRK for AT2R) and angiotensin-converting enzyme (kidney extract for ACE). The second lane was loaded with pre-stained molecular weight standards (Wt std). The next two lanes had biopsy material from squamous epithelium of a control subject (Sq ctr, lane 3) and from one BE patient (Sq BE, lane 4). Lane 5 had biopsy material from metaplastic epithelium in a BE patient (Mtp BE).

A strong immunoreactivity to ACE was noted in the vessel walls of all BE samples. Four out of eight BE-HGD patient samples showed several areas with very distinct staining for ACE in the surface epithelial cells (Figure 5(c)), which was never observed in any of the samples from BE-ND patients (Figure 4(c)).

Discussion

In addition to its classical endocrine character described in textbooks, the RAS has complex tissue-based synthesis pathways and several targets for functional regulation. Although a number of bioactive angiotensins occur following degradation of the pro-hormone angiotensinogen, the classical mediator AngII is regarded as the primary effector of the RAS.[26] To restrict the analysis of the present investigation, we concentrated on ACE as a good representative of AngII formation capability, and on the AngII receptors AT1R and AT2R. The AT1R is of particular interest because it exerts pro-inflammatory and trophic effects and may even be pro-neoplastic. This feature has not previously been investigated in BE, but epidemiological studies have shown a lower incidence of esophageal cancer in patients treated with ACE inhibitors and AT1R blockade, suggesting a link to mucosal RAS activity.[17] The present investigation showed a significantly altered protein expression, with AT1R being higher and ACE being lower in the squamous epithelium of BE patients than in control subjects of similar age. These findings make it tempting to suggest that the transformation from a phenotype with normal squamous cell-lined esophagus to abnormal conditions (i.e., esophagitis, BE, or even carcinoma) initially involves

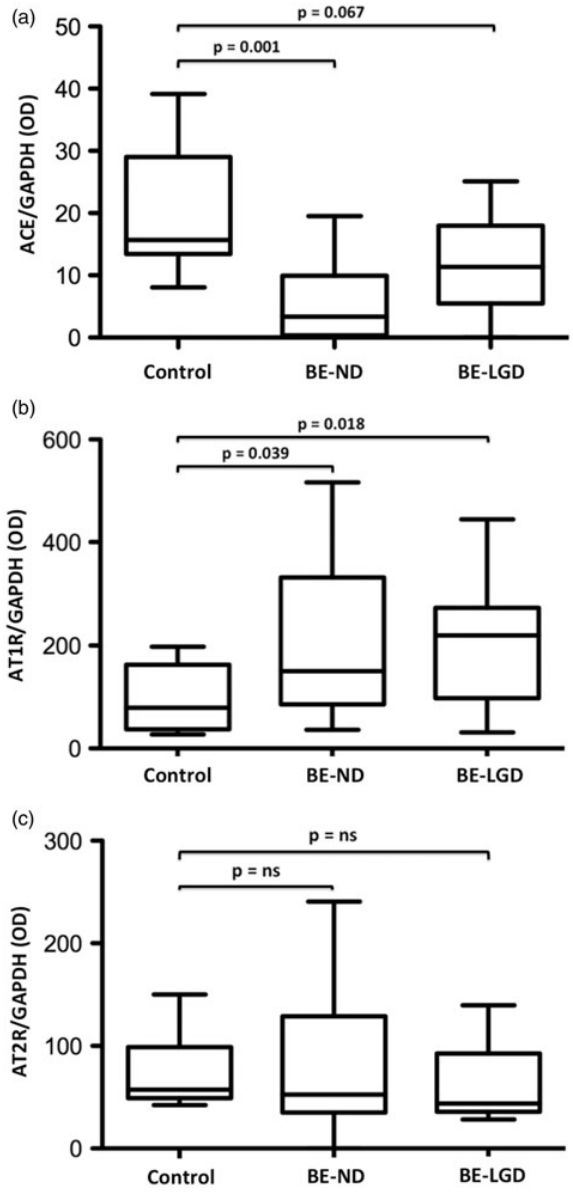

Figure 2. Box-and-whisker plots showing results of western blotting regarding ACE (panel a), AT1R (panel b), and AT2R (panel c) in biopsies from squamous epithelium in controls ($n = 7$) and in BE patients who were either diagnosed as non-dysplastic (BE-ND, $n = 26$) or having low-grade dysplasia (BE-LGD, $n = 16$). Data are optical density (OD) relative to GAPDH as housekeeping protein. Median values are indicated by the transverse line within the box, the interquartile range by the height of the box, and 5th and 95th percentiles by the ends of the whiskers. Mann–Whitney *U* test.

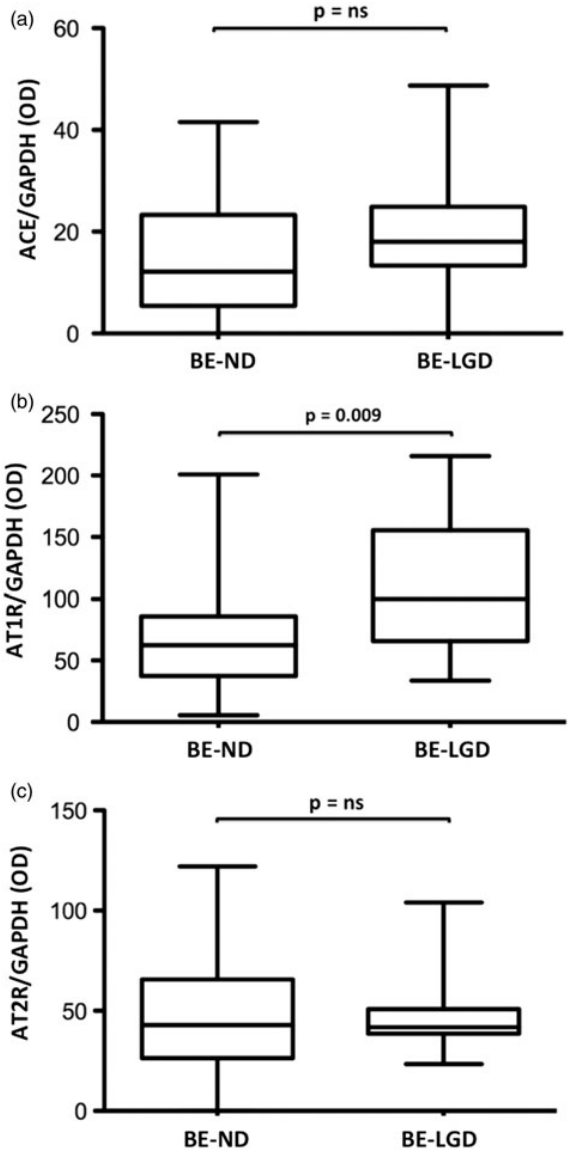

Figure 3. Box-and-whisker plots showing results of western blotting for protein expression of ACE (panel a), AT1R (panel b), and AT2R (panel c) in biopsies from BE patients with no dysplasia (BE-ND, $n = 26$) or low-grade dysplasia (BE-LGD, $n = 16$). Data are optical density (OD) relative to GAPDH as housekeeping protein. Median values are indicated by the transverse line within the box, the interquartile range by the height of the box, and 5th and 95th percentiles by the ends of the whiskers. Mann–Whitney *U* test.

suppressed expression of ACE and increased induction of the pro-inflammatory protein AT1R. In a previous study performed in our laboratory, patients with gastro-esophageal reflux disease showed increased expression AT2R protein.[20] This contrasts with the squamous mucosa of the BE patients in the present investigation, which did not differ significantly from non-BE controls regarding AT2R expression. This is an interesting pattern, considering the postulated tissue-protective function of AT2R by inhibiting functions of AT1R. One could speculate that a defective expression of AT2R would result in an uncontrolled AT1R-driven chronic pro-inflammatory signalling that contributes to metaplastic and possibly dysplastic mucosal transformation.

The western blot assessments of mucosa from BE patients diagnosed with dysplasia showed significantly higher levels of AT1R than in BE patients without dysplasia. The association of AT1R with dysplasia suggests that it may have a role in the pre-neoplastic phase of carcinogenesis. It is of interest to note that the topographical distribution of AT1R was less abundant in patients with dysplasia, whereas ACE was more widely distributed in the surface epithelium of these patients (Figure 5). The latter observation is of interest because it is known that the degree of ACE-expression is related to the state of differentiation of the enterocytes.[27]

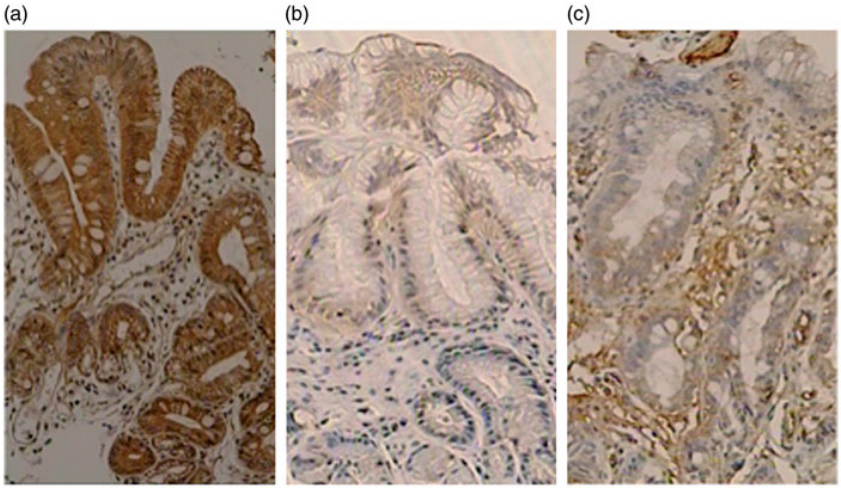

Figure 4. Cross-section of esophageal mucosa from BE patient with no dysplasia stained with: anti-AT1R antibody (a), anti-AT2R antibody (b), and anti-ACE antibody (c). Background staining with haematoxylin and eosin.

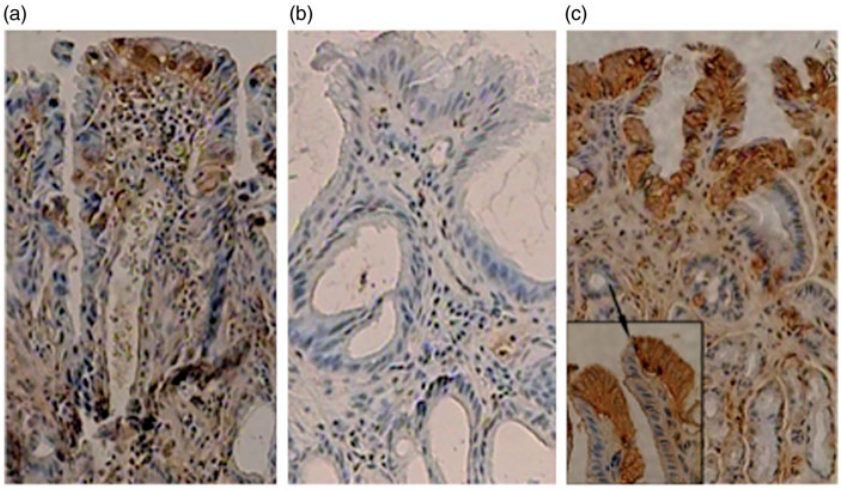

Figure 5. Cross-section of esophageal mucosa from BE patient with high-grade dysplasia stained with: anti-AT1R antibody (a), anti-AT2R antibody (b), and anti-ACE antibody (c). Background staining with haematoxylin and eosin. (Arrow in image miniature in panel c shows cell clones with strong staining adjacent to unstained epithelial cells.)

At present it is not possible to conclude that there is any distinct pathophysiological effect of the aberrant expression of the RAS components in association with dysplasia in BE. RAS is a potent regulatory super-system and the present demonstration of its presence in the epithelium of the human esophageal mucosa paves the way for future research. AngII has been shown to regulate the functional state of small intestinal mucosal enterocytes.[28] Perhaps the well-established potential of AngII receptors to influence cellular growth and differentiation may also be operational in BE, including modulation of inflammation and participation in carcinogenesis.[29] Future studies are also needed to address the possibility of using ACE and AngII receptors as biomarkers for BE-associated carcinogenesis. Another exciting future research possibility is pharmacological interference using pharmaceuticals already on the market, for example AT1R antagonists and ACE inhibitors.

The present study had a number of limitations. The clinical study population was small and unsorted, giving a risk for selection bias and of weaknesses in the association analyses. On the other hand, no attempts were made to influence the distribution of cases in the various assessments, supporting the idea that the results truly reflect conditions in BE patients. The study cohorts were subdivided according to previously obtained histopathological diagnoses. However, it is well known that dysplastic changes in Barrett mucosa have a patchy distribution.[30] Thus, the mucosal samples investigated in the present study were not confirmed as having (or not having) dysplasia. To reduce variability, the taking of biopsies was carefully standardised to the 3 o'clock position where the majority of mucosal erosions in patients suffering from esophagitis occur,[31] being a site also at risk of developing adenocarcinoma, as demonstrated by Cassani et al.[32] In summary, western blotting indicated lower ACE expression and higher AT1R expression in the remaining squamous mucosa of BE patients than in mucosa of non-BE controls. In mucosa from intestinal metaplasia, AT1R expression was elevated in BE patients with confirmed dysplasia. The differential

expression of RAS components in the esophageal mucosa of BE patients is intriguing, and suggests an association with the pro-neoplastic progression. The extent to which this is a cause or a consequence remains to be investigated.

Acknowledgements

Technical assistance by Christina Ek, My Engström, Gunilla Bogren and Eva Een is gratefully acknowledged and we thank Sören Lundberg for reviewing the statistics.

Disclosure statement

The authors have no conflicts of interests.

Funding information

This study was supported by grants from the Swedish Research Council (VR medicine), the Gothenburg Medical Society, the ALF agreement of the Western Region (VGR), and the Capio Research Fund.

References

[1] Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst. 2005;97:142–146.
[2] Voutilainen M. Epidemiological trends in oesophageal cancer in the Nordic countries. Scand J Gastroenterol. 2008;43:323–327.
[3] Pohl H, Sirovich B, Welch HG. Esophageal adenocarcinoma incidence: are we reaching the peak? Cancer Epidemiol Biomarkers Prev. 2010;19:1468–1470.
[4] Kapoor H, Agrawal DK, Mittal SK. Barrett's esophagus: recent insights into pathogenesis and cellular ontogeny. Transl Res. 2015;166:28–40.
[5] von Bohlen und Halbach O, Albrecht D. The CNS renin–angiotensin system. Cell Tissue Res. 2006;326:599–616.
[6] Lazartigues E, Feng Y, Lavoie JL. The two fACEs of the tissue renin–angiotensin systems: implication in cardiovascular diseases. Curr Pharm Des. 2007;13:1231–1245.
[7] Weir MR, Dzau VJ. The renin–angiotensin–aldosterone system: a specific target for hypertension management. Am J Hypertens. 1999;12:205S–213S.
[8] Leung PS. Local renin–angiotensin system in the pancreas: the significance of changes by chronic hypoxia and acute pancreatitis. Jop. 2001;2:3–8.
[9] Leung PS. The peptide hormone angiotensin II: its new functions in tissues and organs. Curr Protein Pept Sci. 2004;5:267–273.
[10] Hirasawa K, Sato Y, Hosoda Y, et al. Immunohistochemical localization of angiotensin II receptor and local renin–angiotensin system in human colonic mucosa. J Histochem Cytochem. 2002;50:275–282.
[11] Suzuki Y, Ruiz-Ortega M, Lorenzo O, et al. Inflammation and angiotensin II. Int J Biochem Cell Biol. 2003;35:881–900.
[12] Weber KT. Fibrosis, a common pathway to organ failure: angiotensin II and tissue repair. Semin Nephrol. 1997;17:467–491.
[13] Deshayes F, Nahmias C. Angiotensin receptors: a new role in cancer? Trends Endocrinol Metab. 2005;16:293–299.
[14] de Gasparo M, Catt KJ, Inagami T, et al. International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev. 2000;52:415–472.
[15] de Gasparo M, Siragy HM. The AT2 receptor: fact, fancy and fantasy. Regul Pept. 1999;81:11–24.
[16] Fandriks L. The renin–angiotensin system and the gastrointestinal mucosa. Acta Physiol (Oxf). 2011;201:157–167.
[17] Sjoberg T, Garcia Rodriguez LA, Lindblad M. Angiotensin-converting enzyme inhibitors and risk of esophageal and gastric cancer: a nested case-control study. Clin Gastroenterol Hepatol. 2007;5:1160–1166.
[18] Casselbrant A, Edebo A, Wennerblom J, et al. Actions by angiotensin II on esophageal contractility in humans. Gastroenterology. 2007;132:249–260.
[19] Casselbrant A, Edebo A, Hallersund P, et al. Angiotensin II receptors are expressed and functional in human esophageal mucosa. Am J Physiol Gastrointest Liver Physiol. 2009;297:G1019–G1027.
[20] Bjorkman E, Edebo A, Casselbrant A, et al. The renin–angiotensin system in the esophageal mucosa of healthy subjects and patients with reflux disease. Scand J Gastroenterol. 2013;48:147–159.
[21] Miwa H, Hongo M, Kusano M. Combination of angiotensin II receptor blockers promotes proton pump inhibitor-based healing of reflux esophagitis. J Gastroenterol. 2012;47:249–255.
[22] Lau ST, Leung PS. Role of the RAS in pancreatic cancer. Curr Cancer Drug Targets. 2011;11:412–420.
[23] Carlsson R, Dent J, Bolling-Sternevald E, et al. The usefulness of a structured questionnaire in the assessment of symptomatic gastroesophageal reflux disease. Scand J Gastroenterol. 1998;33:1023–1029.
[24] Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. Gut. 2000;47:251–255.
[25] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem. 1976;72:248–254.
[26] Kurdi M, De Mello WC, Booz GW. Working outside the system: an update on the unconventional behavior of the renin–angiotensin system components. Int J Biochem Cell Biol. 2005;37:1357–1367.
[27] Naim HY. Secretion of human intestinal angiotensin-converting enzyme and its association with the differentiation state of intestinal cells. Biochem J. 1996;316:259–264.
[28] Carey RM, Levens NR, Peach MJ. Regulation of intestinal fluid transport by angiotensin II: mechanisms and physiological significance. Trans Am Clin Climatol Assoc. 1984;95:93–104.
[29] Smith GR, Missailidis S. Cancer, inflammation and the AT1 and AT2 receptors. J Inflamm (Lond). 2004;1:3 doi: 10.1186/1476-9255-1-3.
[30] Sharma P, Weston AP, Topalovski M, et al. Magnification chromoendoscopy for the detection of intestinal metaplasia and dysplasia in Barrett's oesophagus. Gut. 2003;52:24–27.
[31] Edebo A, Vieth M, Tam W, et al. Circumferential and axial distribution of esophageal mucosal damage in reflux disease. Dis Esophagus. 2007;20:232–238.
[32] Cassani L, Sumner E, Slaughter JC, et al. Directional distribution of neoplasia in Barrett's esophagus is not influenced by distance from the gastroesophageal junction. Gastrointest Endosc. 2013;77:877–882.