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The role of Angiotensin II type 1a receptor on intestinal epithelial cells following small bowel resection in a mouse model

Hiroyuki Koga, MD^{*}, Hua Yang, MD^{†,*}, Emir Q Haxhija, MD^{*,‡}, and Daniel H Teitelbaum, MD^{*}

^{*}Section of Pediatric Surgery, Department of Surgery University of Michigan, Ann Arbor, Michigan, USA

[†]Department of General Surgery, Xinqiao Hospital, Chongqing, China

[‡]Department of Pediatric Surgery, Medical University Graz, Graz, Austria

Abstract

Aim—We have previously shown that inhibition of angiotensin converting enzyme (ACE) significantly reduced intestinal epithelial cell (EC) apoptosis and improved morphometric intestinal adaptation in a mouse model of massive small bowel resection (SBR). This study attempted to further examine the downstream signaling factors in this system by blocking the action of angiotensin II (ATII), hypothesizing that this would lead to similar improvement of intestinal adaptation after SBR.

Method—Two groups of mice (C57BL/6J) underwent either a 60 % mid-intestinal resection (SBR group) or a transaction/re-anastomosis (Sham group). Because real-time PCR studies showed that only ATII receptor type 1a (ATII-1a) expression was significantly increased after SBR, compared to SHAM mice, we decided to use the specific ATII-1a receptor antagonist Losartan, to block this signaling pathway. An additional two groups of mice received daily i.p. injections of Losartan (SBR +Losartan and Sham+Losartan group). At 7 days the adaptive response was assessed in the remnant gut including: villus height, crypt depth, EC apoptosis (TUNEL staining) and proliferation (BrdU incorporation). The apoptotic and proliferation signaling pathways were addressed by analysis of EC mRNA expression.

Result—SBR (with and without Losartan) led to a significant increase in villus height and crypt depth. Losartan treatment did not significantly change EC proliferation, but did significantly reduce EC apoptosis rates as compared to the non-treated SBR group. Losartan treatment was associated with a significant reduction of the bax-to-bcl-2 ratio and TNF- α expression after SBR compared to non treated groups. Interestingly, Losartan treated groups showed a tremendous increase in proliferation signaling factors EGFR, KGFR and IL7R, which may indicate an expanded potential for further intestinal adaptation also beyond 7 days after SBR.

Conclusion—This study showed that the ATII-1a receptor may be of crucial importance for the modulation of intestinal EC apoptosis and proliferation, and for regulating the post-resectional EC adaptive response.

Keywords

Renin-angiotensin-system; angiotensin II receptor type 1a (ATII-1a); small bowel resection

INTRODUCTION

Short bowel syndrome (SBS) is characterized as a state of maldigestion and malabsorption, and may occur with congenital anomalies or after massive small bowel resection (SBR). SBR leads to an adaptive response in residual intestine. Following SBR the residual intestine undergoes a series of adaptive processes in order to increase absorptive surface area and establish nutritional homeostasis. The exact mechanisms of post-resectional intestinal adaptation are incompletely understood, although a number of nutritive and nonnutritive factors have been identified as potential mediator of this compensatory intestinal response^{1–3}. The adaptive processes which occur in the residual small intestine after SBR include marked changes in epithelial cell (EC) proliferation and enterocyte apoptosis rates^{4–9}; which under normal physiologic circumstances are balanced, and shifts in favor of compensatory mucosal growth. Increases in EC proliferation may well occur via an up-regulation in a number of growth factors including epidermal growth factor (EGF) expression and EGF receptor (EGFR)^{10, 11}; as well as a number of other growth factors including keratinocyte growth factor (KGF)¹², IL-7¹³, transforming growth factor alpha¹⁴, and glucagons like peptide 2¹⁵.

An increase in the ratio between pro-apoptotic and anti-apoptotic factors has also been reported after massive SBR¹⁶. It is well documented that the bcl-2 family has an important role in the regulation of intestinal EC apoptosis via the intrinsic apoptotic pathway^{17–19}. Two additional factors which mediate EC, tumor necrosis factor-alpha (TNF- α) and Fas, have also been shown to be up-regulated after SBR in rodents^{4, 5, 20}. These factors may therefore also be involved in the regulation of post-resectional EC apoptosis in the frame of the extrinsic apoptosis pathway.

The rennin-angiotensin-system (RAS) exerts multiple biological functions including cell growth, physiologic maintenance of blood pressure and perfusion, as well as mediation of inflammation contributing to the progression of tissue damage^{21, 22}. Pharmacologic blockade of the actions of Angiotensin II (ATII) are known to have the beneficial effects of reduced inflammation in several organ systems (e.g., vasculature, kidney and liver)^{21, 23, 24}; however, the function of the RAS in the intestine is not well understood. Our laboratory has previously shown that ACE is expressed at particularly high levels in intestinal epithelium, and to be critically important in promoting the development of intestinal EC apoptosis^{20, 25, 26}. The effect of ACE and ATII are mediated through a series of cell surface ATII receptors, and interestingly, these receptors are also found in the intestinal mucosa²⁷. Our recent studies using pharmacologic ACE inhibitors (ACE-I) have shown that ACE-I significantly reduces EC apoptosis and moderates enhancement of intestinal adaptation (increased crypt depth and increased EC proliferation) after massive SBR²⁰. Other investigators have reported that RAS mediation of apoptosis is via ATII signaling through its respective receptors in a number of organs and cells such as lung, prostate and endothelial cell^{28–30}. Further, blockade of ATII by ACE-I or ATII receptor antagonists reduces injury effects and apoptosis, findings that were closely correlated to the reduction of TNF- α expression^{23, 24, 31, 32}. Taken together, these findings suggested that ATII is a central mediator in the pathogenesis of intestinal adaption after massive SBR, and neutralization of ATII could be a beneficial therapeutic target through a reduction of this pro-apoptotic factor in the residual intestine.

In this study, we hypothesized that the ATII antagonist would influence post-resectional intestinal adaptation through changes in EC apoptosis and proliferation in an experimental model of SBS after massive SBR. Namely, antagonisms of ATII signaling would result in decreased EC apoptosis and increased intestinal adaptation after formation of SBS. To approach this question, we firstly investigated the expression of ATII receptor in intestine after massive SBR, and then examined the mechanisms in the process of enterocyte apoptosis and proliferation in this model.

MATERIAL and METHODS

Animal

Specific pathogen free, 8 week old male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were maintained in a 12-h night rhythm at 23°C and a relative humidity of 40–60%. A standard rodent chow (LabDiet® 5001 Rodent Diet, PMI Nutrition International, LLC, Brentwood, MO) was switched to microstabilized rodent liquid diet (TestDiet, Richmond, IN) 2 days prior to surgery, and mice were maintained thereafter on liquid diet until harvest. All experiments reported here conformed to the guidelines for care and use of laboratory animals established by the University Committee on Use and Care of Animal at the University of Michigan, and protocols were approved by this Committee.

Experimental design

To investigate the post-resectional change in the role of the RAS, we firstly evaluated the expression of angiotensin II receptor on EC using real-time PCR in SBS and Sham model, and then examined potential mechanistic pathways. Four study groups were used: SBR, 60% mid-small bowel resection without Losartan treatment; SBR+ATII-1a, SBR mice given daily Losartan; Sham, transaction and reanastomosis without Losartan; and Sham+ATII-1a, Sham mice given daily Losartan.

Surgery—A 60% mid-small bowel resection was performed similar to that previously described²⁰. Mice were anesthetized, a median laparotomy created, small bowel was either resected (SBR group) or small bowel transaction was performed (Sham-group). End-to-end anastomoses were performed with interrupted 8–0 monofilament sutures. The abdominal wall was closed in two layers. At the end of surgery mice were resuscitated with a 3 ml subcutaneous injection of warmed saline and allowed free access to water and liquid diet. Body weights were determined preoperatively and at harvest.

Treatment with AT II receptor antagonist

The ATII receptor type 1a (ATII-1a) antagonist Losartan, (10mg/kg/day i.p. Merck co, West point, PA) was given to a separate group of Sham and SBS mice starting one day prior to the surgery. In some experiments, mice were matched to a control group of non-operated mice; and were studied after a 7-day period.

Harvesting

Mice were euthanized 7-days after surgery using carbon dioxide asphyxiation. The small bowel (0.5cm) segments were preserved in 10% buffered formalin. Jejunal and ileal tissues were taken 3cm distal to ligament of Treitz and 3cm proximal to the ileocecal junction. Small bowel within 0.5 cm of the anastomotic sites was discarded. The remaining small bowel was immediately processed for mucosal cell isolation.

Histology

A 0.5-cm segment do mid-small bowl (at least 2 cm away from anastomosis) was fixed in 10% formaldehyde, and processed for hematoxylin and eosin staining. Villus height and crypt depth were measured using a calibrated micrometer. Each measurement consisted of a mean of measures derived from 16 different low power fields.

Epithelial cell proliferation

To examine the changes in intestinal crypt cell proliferation rate 5-bromo-2-deoxyuridine (BrdU) as an active cell division marker was used, as described previously^{20, 25}. Mice were injected intraperitoneally with BrdU (50mg/kg, Roche Diagnostic, Indianapolis, IN) 1-hour

before harvest. Paraffin-embedded section of 5 μm thickness were stained using BrdU In Situ Detection Kit II according to the manufacturer's guideline (BD PharMingen, San Jose, CA). An index of crypt cell proliferation rate was calculated using the ratio of the number of crypt cells incorporating BrdU to the total number of crypt cells. The total number of proliferating cells per crypt was defined as a mean ratio of proliferating cells in 16 well oriented crypts (counted at $\times 400$ magnification).

Epithelial cell apoptosis assays

A terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL) staining method was used for EC apoptosis assay, according to manufactures's instructions (ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit, Chemicon International, Temecula, CA), with slight modification, as described previously^{20, 25}. Assessment of apoptosis consisted of separate counting of all TUNEL positive EC in all well-oriented crypts and villi separately and dividing the total number of counted apoptotic cells per number of analyzed crypts and villi, respectively. Adding together of both crypt and villus apoptotic indices is expressed as the apoptotic index per crypt-villus complex (at $40\times$ magnification).

Mucosal cell isolation and purification

Isolation of mucosal cells was performed using a previously described protocol³³.

Real time polymerase chain reaction (RT-PCR)

Real time PCR was performed from isolated RNA based on previous standard protocols using the $\Delta\Delta\text{Ct}$ method²⁰. All primers for selected gene sequences were designed using proprietary software (Lasergene, DNA star Inc, Madison, WI). Real-time PCR (RT-PCR) was performed using a Smart Cycler (Cepheid, Sunnyvale, CA) with intercalation of SYBR green I used to determine the amount of DNA using previously published techniques³³. Expressions of result were normalized to β -actin expression.

Measurement of Mucosal and Mesenteric Blood Flow

To evaluate the effect of ATII-1a blockade on mesenteric blood flow, the laser Doppler perfusion imager (LDPI; Perimed Inc, North Royalton, OH) with computer software was used as previously described²⁶. Briefly, in anesthetized mice (Naïve and Naïve+ATII-1a, $n=5$, in each group) a median laparotomy was performed and the mesentery exposed. A 670 nm helium-neon laser beam was placed 12-cm above the mesentery to sequentially scan the surface of mesentery and detect moving blood cells. Maximum, minimum, and mean percent perfusion was normalized to total pixel area. At the end of the measurement the mice were euthanized.

Statistical analysis

Data are reported as mean $\pm$ standard deviation (SD). Results were analyzed using t test for comparison of two means, and a one-way analysis of variance (ANOVA) for comparison of multiple groups. A post-hoc Bonferroni test was used to assess statistical difference between groups. The chi square test was used for categorical data (Prism software; GraphPad Software, Inc., San Diego, CA). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Changes in AT II receptors expression after bowel resection

Increased ATII receptor type 1a (ATII-1a) after bowel resection—Real time-PCR showed a significantly increased expression of ATII-1a mRNA in the SBR group when compared to the Sham group ($P < .05$, $N=6$) (Table 1). And there were no significant changes in the expression of other receptors.

Effect of ATII-1a blockade on small intestine epithelium

To understand the significance of increased altered ATII-1a expression on SBS adaptive changes, the ATII-1a antagonist, Losartan was given to a separate group of Sham and SBR mice. These mice were matched to an untreated (non-operated) group, and were studied after a 7-day period. All mice tolerated surgery well and showed no signs of intestinal obstruction at harvest.

Weight changes in the SBS model—Both Sham and SBR mice showed some degree of weight loss following surgery; however, the loss in each of these plateaued by the latter two days of the post-surgery period. The loss of weight in the SBR group was significantly greater than the Sham (percent change from weight at surgery: $-18.5 \pm 2.2\%$ versus $-10.5 \pm 1.5\%$, respectively; $P < .001$). However, this loss was significantly attenuated in the SBR+ATII-1a group ($-7.6 \pm 0.9\%$, $P < .001$). There was no significant difference in weight loss between the Sham+ATII-1a group versus the Sham group ($-4.2 \pm 1.8\%$, $P > .05$).

ATII-1a blockade significantly altered mucosal morphometry—The adaptive responses after SBR are shown in Table 2. Both, villus height and crypt depth significantly increased after SBR as compared to Sham group. Losartan administration to SBS mice (SBS +ATII-1a) resulted in a significant increase in villus height, but failed to influence the increase in crypt depth beyond that seen in untreated SBS mice. Those mice undergoing a sham resection and treated with ATII-1a blockade (Sham+ATII-1a) showed no significant alteration in histological features in either crypt depth or villus height when compared with the Sham group.

Effect of AT II receptor type 1a antagonist on epithelial apoptosis

Enterocyte apoptosis rates were significantly higher in the SBS group compared to Sham group (Table 2), in agreement with previous studies^{4, 20, 34}. Administration of an ATII-1a antagonist resulted in a significant decline in EC apoptosis rates in both the SBS and Sham groups. EC apoptosis decreased five-fold when compared with the untreated SBS group. These levels declined to those similar to that observed in the Sham group mice.

Mechanism of ATII receptor type 1a antagonist-mediated EC apoptosis

To investigate this mechanisms which led to the decline in EC apoptotic rates with Losartan, several factors in the both the intrinsic (bcl-2 family) and extrinsic (TNF- α and Fas) components of apoptotic signaling were investigated.

Intrinsic pathway—The mRNA expression of anti-apoptotic factor bcl-2, a member of the bcl-2 family, showed a significant increase in the SBR group compared to Sham group ($P < .001$) (Figure1). The expression of bcl-2 significantly decreased with Losartan treatment in SBR mice ($P < .05$). Interestingly, Losartan treatment did not significantly affect Sham control mice. The expression of the pro-apoptotic factor bax increased significantly after SBR group compared to Sham group ($P < .05$) (Figure1). ATII-1a blockade led to a significant decline in bax expression in SBR+ATII-1a group ($P < .05$).

Extrinsic pathway—Gene expression of the investigated members of the extrinsic apoptotic pathways (TNF- α , TNF receptor 1 (TNFR1), Fas and FasL) was performed next. Interestingly, SBR led to an increased expression of TNF- α , TNFR1, Fas and although not significant, an increase in FasL. Mice treated with Losartan showed a similar increase compared to Sham mice in each of these factors (Figure 2). SBR mice treated with Losartan did show a significant decline in TNF- α compared to the untreated SBR group; however the level of TNF- α remained significantly higher than Sham+ATII-1a mice.

Effect of ATII receptor type 1a antagonism on epithelial cell proliferation

To better understand the implication of AT II-1a blockade on the SBR adaptation, EC proliferation was assessed with BrdU incorporation (Table 2). SBR led to a significantly increased level of proliferation compared to Sham mice. Losartan treatment, however, increased this level of proliferation, and levels of proliferation were actually higher ($P<0.05$) in Losartan treated mice compared with untreated SBR mice.

Mechanisms of ATII receptor type 1a antagonism-mediated epithelial cell proliferation

To investigate the mechanisms which resulted in the differences in EC apoptosis and proliferation rates, mRNA expressions of KGFR₁, IL-7R and EGF-R were studied. We found that administration of ATII-1a blockade led to a markedly increased expression of each of these growth factor receptors. Further this increase was noted in both Sham and SBR mice. However, Losartan treatment was not significantly higher in the SBR mice compared to the Sham controls.

Effect of ATII receptor type 1a blockade on intestinal blood flow

Mesenteric blood flow measurements showed slightly lower levels in ATII-1a treated group compared to untreated group; however, the values were not significantly different between the two groups (2.7 ± 0.3 versus 2.5 ± 0.1 , respectively; expressed in arbitrary units).

DISCUSSION

In this study, we demonstrated several findings. First, the increase in EC-derived ATII type 1a receptor expression after formation of SBS was confirmed with real-time PCR. We hypothesized that this increase in EC-derived ATII type 1a receptor may be associated with the intestinal EC adaptive process after SBR. To determine the functional role of angiotensin II signaling via the ATII type 1a receptor in the intestinal mucosa, we used the ATII-1a specific antagonist, Losartan. Losartan treatment significantly decreased EC apoptosis and increased epithelial proliferation in the small intestine after SBR. This suggested a potential mechanism for SBR adaptation, and a mode of treatment which could be used to augment this adaptation. Additionally, our study showed that secondary formation of intestinal adaptation after massive SBR was associated with increased expression of TNF- α , bcl-2 and bax, and these factors may well play an important role in the development of adaptation^{4, 20, 25}. Finally, treatment with Losartan led to a change in the expression of several pro-apoptotic signaling factors including a decline in TNF- α , Bax and Bcl-2; suggesting that inhibition of the TNF- α signaling pathway is a potential mechanism for the prevention of intestinal apoptosis.

ATII has been identified as the principal mediator of RAS function. ATII may mediate its action via at least two receptors ATII type 1 (a and b) and type 2 which are expressed by various cells, including immune mediating cells (e.g., T-cells and macrophages). Several investigators have shown that ATII is implicated as promoters of apoptosis in other organ systems^{28, 29, 35}. ATII, produced by ACE, is necessary for the mediation of apoptosis in pulmonary and vascular smooth muscle cells^{21, 31}. Although it has been demonstrated that RAS is highly expressed in small and large intestine of rodents and humans^{27, 36}, and our previous studies have demonstrated that RAS has an important role in intestinal adaptation after SBR^{20, 25}; however, it is not understood how ATII mediates this action these adaptive actions. Based on our finding of increased ATII-type 1a receptor expression on intestinal mucosa after SBR, our laboratory investigated the role of ATII-1a in intestinal adaptation using a SBR model.

ATII-1a signaling is known to clearly affect the blood flow and blood pressure. In order to rule out the possibility that major alternations in mesenteric blood flow could account for the observed changes in apoptosis and proliferation. Doppler blood flows were determined. We

found no significant change in blood flow between ATII-1a treated and untreated mice, suggesting that alterations in blood flow had little to no effect on the ATII-1a associated adaptation.

After massive SBR intestinal adaptation is structurally characterized by an increase in EC proliferation and EC apoptosis rate. Although mechanisms leading to increased EC apoptosis after SBR are incompletely understood, the pro-apoptotic protein bax and other members of the intrinsic apoptotic pathway play a crucial role^{4–7, 9, 16, 37}; as well, regulatory linkage between the intrinsic and extrinsic apoptotic pathway has also been speculated⁵. Our findings of an increase in several of these regulatory factors in the present study are consistent with previous reports^{4, 5, 9, 38}, supporting both apoptotic pathways in the regulation of post-resectional EC apoptosis. In contrast, the administration of ATII-1a blockade (Losartan) showed a similar increase in expression of bcl-2 but an unchanged expression of bax after SBR. This led to significant reduction in the bax to bcl-2 ratio in Losartan treated mice; and this ratio of factors has been shown to be a valuable indicator for modulating the development of apoptosis^{4, 6, 16, 37}. Recent studies have linked apoptosis mediated by the intrinsic pathway to the local RAS; and the mediation of apoptosis appears to be through the bcl-2 family, including a decrease in the bax to bcl-2 ratio^{39–41}. Blockade of ATII signaling, via either an ACE-inhibitor or selective blockade of ATII receptors have been shown to effectively reduce apoptosis rates in various tissue^{20, 39, 40}. Another potential mechanism of the increase in post-SBR EC apoptosis may be via TNF- α . In the present study intestinal mucosa expression of TNF- α was increased after SBR, and administration of Losartan significantly reduced this expression. This down-regulation of TNF- α , suggests that this may be a mechanism by which blockade of ATII-1a reduced apoptosis. Further, this finding is similar to the results of other investigators examining mediation of apoptosis in cardiac and pulmonary tissue^{42, 43}.

Another signaling pathway for EC apoptosis is via Fas/Fas-ligand (FasL) signaling. In the present study we did detect an increase in either Fas or Fas-L mRNA expression after SBR; as well, this was not influenced by the administration of Losartan. This finding is distinct from other investigators who showed that activation of Fas stimulates alveolar EC to synthesize ATII *de novo*⁴⁴. These same investigators also showed that the autocrine synthesis of ATII was found to be required for the induction of apoptosis via Fas, and apoptosis could be abrogated by ACE-inhibitor or selective blockade of ATII receptors⁴⁴. Our findings suggest, at least within the intestinal mucosa, that the Fas/Fas-L extrinsic pathway is not involved to RAS in SBR associated EC apoptosis.

SBR mice showed significantly higher EC proliferation rates compared with Sham mice. Additionally, there was a slight increase in EC proliferation and a marked decline in EC apoptosis in the Losartan treated groups. These two changes in the EC was associated with a markedly higher gene expression of the growth factor receptors KGFR₁, EGF-R, IL-7R, compared with the untreated groups. It is possible that the up-regulation of these receptors may well explain the observed increase in EC proliferation^{33, 45–48}; and signaling through several growth factors, including the 3 studied, may also lead to a down-regulation of apoptosis^{47, 49}. These differences in EC apoptosis and proliferation rates were reflected in the intestinal morphology by means of greater crypt depth and villus height in the intestinal epithelium of Losartan treated mice compared to untreated mice. Therefore, we consider that signaling via the ATII receptor type 1a is an important modulator of intestinal homeostasis during intestinal adaptation.

In conclusion, SBR was associated with a previously undescribed increase in the expression of ATII type 1a receptor in the intestinal epithelium. Another novel finding was that administration of the ATII-1a antagonist Losartan reduced SBR-associated EC apoptosis rates and increased EC proliferation; suggesting that ATII-1a signaling may have a similar action

on intestinal epithelial cells as has been previously seen in other organ systems. Possible anti-apoptotic mechanisms of Losartan in the intestinal tract may be through RAS by the reduction in the bax to bcl-2 ratio after SBR; and the proliferative effects may be via an increase in the expression of epithelial cell receptors for several growth factors. It is potentially possible that SBS patients may benefit from use of an ATII-1a antagonist, facilitating the degree of adaptation. Future investigation will hopefully offer greater insight into the action of ATII on the gastrointestinal mucosa.

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Figure 1. mRNA expression of bcl-2 and bax derived from epithelial cell samples in each group and measured by real time PCR. Result are expressed as $2^{-(-\Delta\Delta Ct)}$ in relation to β -actin expression. Expression of both bcl-2 and bax were significantly increased in the SBR group compared to the Sham group. Administration of Losartan (expressed in figure as ATII-1a) to SBR mice significantly reduced the expression of bcl-2 and bax; however, the reduction in bax fell to levels not significantly different than non-treated Sham mice. Results are shown as the mean $\pm$ SD. Statistical comparisons are made using ANOVA with post hoc Bonferroni test.

Figure 2.

mRNA expression of the extrinsic apoptotic pathways as detected by real time PCR. Result are expressed as $2^{-(-\Delta\Delta C_t)}$ in relation to β -actin expression. Note that gene expression of all tested factors were increased in SBR mice, regardless of whether mice received Losartan (expressed in figure as ATII-1a) or not. Results are expressed as mean $\pm$ SD. Statistical comparisons are made using ANOVA with post hoc Bonferroni test.

Figure 3. mRNA expression of EGF-R, KGFR₁, IL-7R. Note the significantly higher expression of these receptors in the Losartan (expressed in figure as ATII-1a) treated mice. Statistical comparisons are made using ANOVA with post hoc Bonferroni test.

Table 1

mRNA expression of Angiotensin II (AT II) receptor in SBS

	ATII receptor, type1a	ATII receptor, type1b	ATII receptor, type2
Sham	1.2±0.2	3.3±0.9	12.6±5.2
SBR	5.0±1.3*	3.5±1.8	18.0±7.3

SBR, Short bowel Resection. ATII, Angiotensin II.

Expression of mRNA Angiotensin II (AT II) receptors on EC as detected by real time PCR. Result are expressed as $2^{-(-\Delta\Delta Ct)}$ in relation to β -actin gene expression. AT II receptor type 1a mRNA expression was significantly increased in the SBR group compared to Sham group ($P \leq 0.05$). Data are expressed as mean $\pm$ SD.

Table 2

Villus-crypt morphology and epithelial cell apoptosis and proliferation rates

	Villus height (μm)	Crypt depth (μm)	Apoptosis index	Proliferation rate
Sham	202±19	100±5	0.05±0.02	20.2±3.2
SBR	312±9 [*]	150±9 [*]	0.11±0.03 [*]	35.2±2.1 [*]
Sham+ATII-1a	221±22	112±8	0.01±0.02	22.6±3.3
SBR+ATII-1a	332±19 ^{**}	148±7 ^{**}	0.03±0.03 ^{**#}	38.6±1.2 ^{**}

Abbreviations: SBR, Short bowel Resection; ATII, Angiotensin II; ATII-1a represents the groups treated with an ATII-1a antagonist (Losartan).

Data are expressed as the mean ± SD. Note significant difference in Villus height and crypt depth between Sham and SBR with/without ATII-1a treatment. Significant post-resectional increases in epithelial cell apoptosis and proliferation rate with/without the ATII-1a antagonist treatment. However, ATII-1a blockade significantly reduced the apoptosis index compared with SBR mice without blockade.

^{*} $P < .05$ Sham vs SBR

^{**} $P < .05$ Sham+ATII-1a vs SBR+ATII-1a

[#] $P < .05$ SBR vs SBR+ATII-1a