Changes in Angiotensin II type 1 receptor expression in the rat bladder by bladder outlet obstruction.

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Abstract

Purpose

To demonstrate the change in the expression of Angiotensin II type 1 receptor (AT1) in the rat bladder with partial bladder outlet obstruction (P-BOO) .

Material and Methods

Bladder specimens were obtained from twelve-week-old Wistar female rats that were divided two groups, a partial bladder outlet obstruction (P-BOO) group and a control group. The rats of the P-BOO group were divided into six groups; sham-operated control group, 1 day postoperatively, 2 days postoperatively, 4 days postoperatively, 7 days postoperatively and 14 days postoperatively. The cystometrical findings and immunohistochemical staining of the detrusor muscle with AT1 antibody was compared in each group.

Results

AT1 localized on the cell membrane of the detrusor smooth muscle and in cytoplasm of suburothelial myofibroblasts in the control rats,. The expression of AT1 disappeared in the detrusor muscle and suburothelial myofibroblasts in P-BOO, but, AT1 was highly expressed

in urothelial cells 1 day after surgery. The expression of AT1 in urothelial cells gradually decreased with time after surgery. AT1 completely disappeared in urothelial cells 14 days after surgery.

Conclusions

The present study demonstrated that the site of AT1 expression changes in response to the mechanical stress caused by P-BOO, and finally there was no expression of AT1 in rat bladder tissue following P-BOO. These data suggest the change in AT1 expression may be play a role in bladder function.

Introduction

Angiotensin II (Ang II) is an important regulatory peptide with multiple physiological functions (1). The main roles of Ang II are contractility, proliferation and fibrosis. The local renin-angiotensin II system (RAS) has been demonstrated in various tissues, including the heart, kidney, adrenal, liver, blood vessels and gonads (2). A few studies investigating the RAS and bladder function were reported in the 90's. They demonstrated that Ang II only has a weak contractive potential, and the blockade of the RAS has little influence on the bladder.

Yamada *et al.* demonstrated that AT1 decreases in the rat bladder with incomplete urinary retention using a bladder outlet obstruction model, and stated that the cause might be down regulation of AT1 induced by the activation of RAS (3).

This study investigated the changes of AT1 expression induced by BOO using immunohistochemistry.

Materials and Methods

Animals

Adult female Wistar rats weighing 200–225 g and about 3 months of age were used in this study. The study protocol was approved by the Animals Ethics Committee at Nagasaki University Graduate School of Biochemical Sciences, Japan. The rats were housed at room temperature with humidity of about 65% and a 12:12 h light: dark cycle. They had free access to water and commercial laboratory chow provided ad libitum. These rats were divided into seven groups; a sham-operated control group, 1 day postoperatively, 2 days postoperatively, 4 days postoperatively, 7 days postoperatively and 14 days postoperatively.

Partial Bladder Outlet Obstruction

A modification of the technique of Mattiasson and Uvelius (4) and Malmgren (5) *et al.* was used to obtain a partial obstruction of the urethra. The rats were anesthetized by

intraperitoneal injection of 1 g/kg body weight urethane. The urethra was intubated with a PE-50 polyethylene catheter and a double 4-0 silk ligature was placed loosely around the proximal urethra producing a standardized degree of obstruction, and the catheter was removed. The control rats underwent a sham operation.

Immunohistochemical staining of AT1

The bladder specimens were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in paraffin. Sections were deparaffinized with xylene and sequentially rehydrated in a graded ethanol series for 5 min each. Endogenous peroxidase activity was blocked by incubation in 3% H_2O_2 in methanol for 15 min. Sections were then incubated at 4°C for 24 hours with rabbit anti-AT1 receptor polyclonal antibody (Santa Cruz, Santa Cruz, CA) diluted 1:500 in phosphate-buffered saline. The sections were washed and incubated with Histofine Simple Stain Rat Max-PO (Nichirei Co., Tokyo, Japan) for 30 min at room temperature. The sections were stained with diaminobenzidine tetrahydrochloride (Nichirei Co., Tokyo, Japan), counterstained with hematoxylin, and mounted.

Cystometric investigation

The rats were anesthetized by intraperitoneal injection of 1 g/kg body weight urethane (6). A PE-50 catheter was inserted through the bladder apex into the lumen and connected to a pressure transducer and a microinjection pump (UD5500, Dantec, Denmark). Warmed saline was then infused into the bladder at a rate of 10 mL/hr for the control and P-BOO rats, respectively.

Results

Cystometric investigation

The voiding pattern in the control rats was normal. The voiding frequency of the P-BOO rats at 1 day and 2 days were significantly greater than that of the control rats. The P-BOO rats at 14 days showed no detrusor contraction and voiding had the characteristics of overflow (**Figure 3**).

Immunohistochemistry of AT1

The localization of AT1 in the rat bladder was examined by immunocytochemistry. **Figure 1** and **2** show the results of the immunohistochemistry. AT1 immunoreactivity was

localized to smooth muscle cells and suburothelial myofibroblasts. AT1 immunostaining in the detrusor muscle was localized predominantly on the cell membrane and slightly in the cytoplasm (**Figure 1, 2**). Replacement of the primary antibody with buffer also yielded negative staining (**Figure 1, 2**). The expression of AT1 in the detrusor muscle and suburothelial myofibroblasts disappeared in P-BOO, but AT1 was highly expressed in urothelial cells at 1 day after surgery, (**Figure 3**). The expression of AT1 in urothelial cells gradually decreased following surgery. There was no ATI immunostaining in the urothelial cells at 14 days after the surgery (**Figure 3**).

Discussion

The present study demonstrated the expression of AT1 on detrusor muscle cells and suburothelial myofibroblasts in the rat bladder. Previous studies demonstrated that Ang II have a potential of contraction of rat bladder, these suggested that AT1 exist on rat bladder tissue. Yamada *et al.* demonstrated that the expression of AT1 in the rat bladder decreases with bladder outlet obstruction using a sensitive binding assay (3). The current data showed the reduction of AT1 expression caused by P-BOO visually and temporally using immunohistochemical staining. Several investigators have shown that Ang II causes pronounced contraction of bladder smooth muscles in some species, including humans (7,8,9,10,11), and the effect is mediated by the AT1. The down-regulation of AT1 in outlet-obstructed rat was observed by Saito *et al.* (12), who showed significantly weaker contractility of human detrusor muscle by Ang II in neurogenic bladders than controls. This suggests that the down-regulation of bladder AT1 is largely ascribable to the enhanced activity of Ang II following P-BOO. Dinh *et al.* reported that dysuria caused by prostatic hyperplasia induces increased expression of Ang II and down-regulation of AT1 in human prostatic tissue (13). The same phenomenon might occur in rat bladder tissue. Pathological conditions of the bladder might reduce expression of AT1, and this mechanism might be the cause of the reduced response of the human bladder to Ang II that Saito *et al.* reported. Although the frequency of urination increased during the period of AT1 expression in urothelial cells, the frequency of urination decreased after AT1 expression disappeared in urothelial cells. AT1 was not expressed in the urothelial layer without mechanical stress by P-BOO. The current data demonstrated that AT1 expression in bladder tissue below the urothelium ceases and new expression of AT1 continues in the urothelium for a few days after acute incomplete urinary retention, and then AT1 gradually disappears from the urothelium. The role of this phenomenon in bladder dysfunction is unknown; however, activated RAS within bladder tissue might induce frequent bladder contraction via AT1 when AT1 is expressed in the urothelium.

Conclusion

The present study demonstrated that AT1 expression in the rat bladder does not uniformly disappear with mechanical stress by P-BOO. The frequency of urination right after the induction of incomplete urinary retention might therefore be associated with the expression of AT1 in the urothelium for a short period of time.

References

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Figure legends

Figure 1. Expression of AT1 on the detrusor muscle in the normal rat bladder. (a) AT1 expressed on the cell membrane of bladder smooth muscle. (b) Negative control Magnificarion ×600

Figure 2. Expression of AT1 in the suburothelial layer in the normal rat bladder. (a) AT1 expressed on suburothelial myofibroblasts. (b) Negative control Magnificarion ×200

Figure 3. The changes of AT1 expression in bladder in response to P-BOO. The expression of AT1 in urothelial cells gradually decreased.

Figure 1.

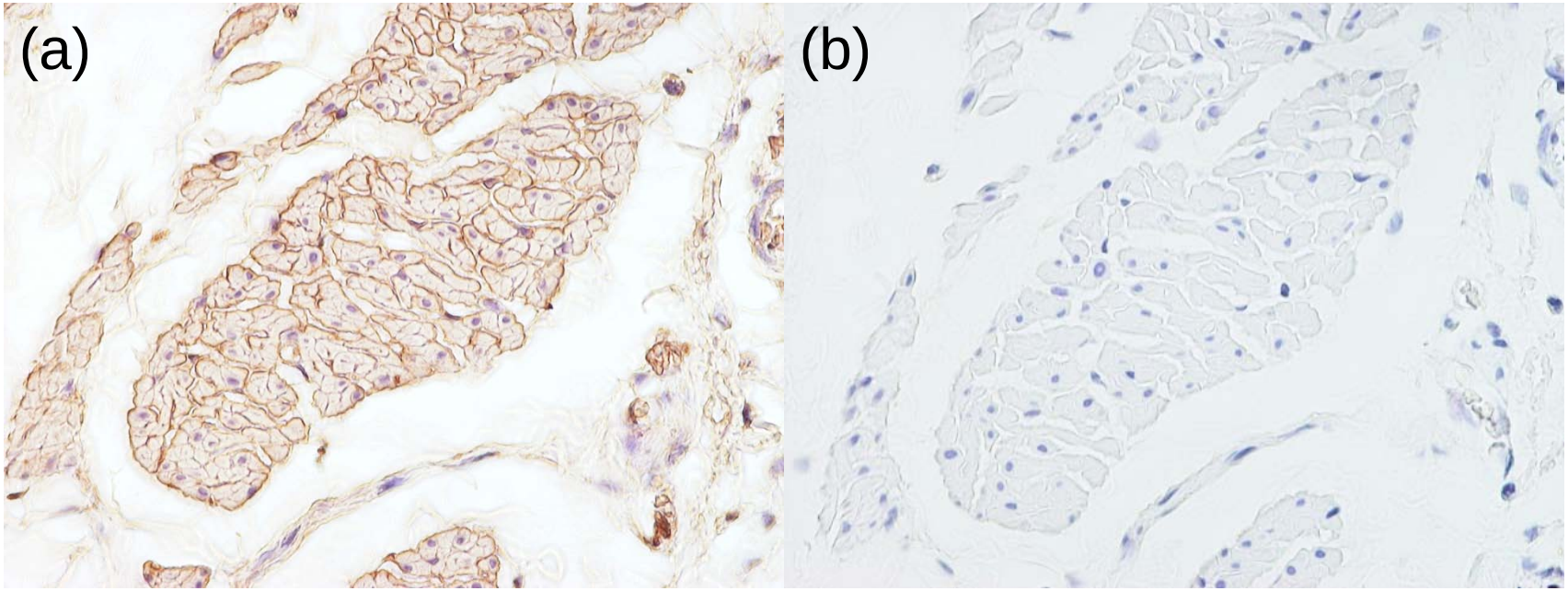

Figure 2.

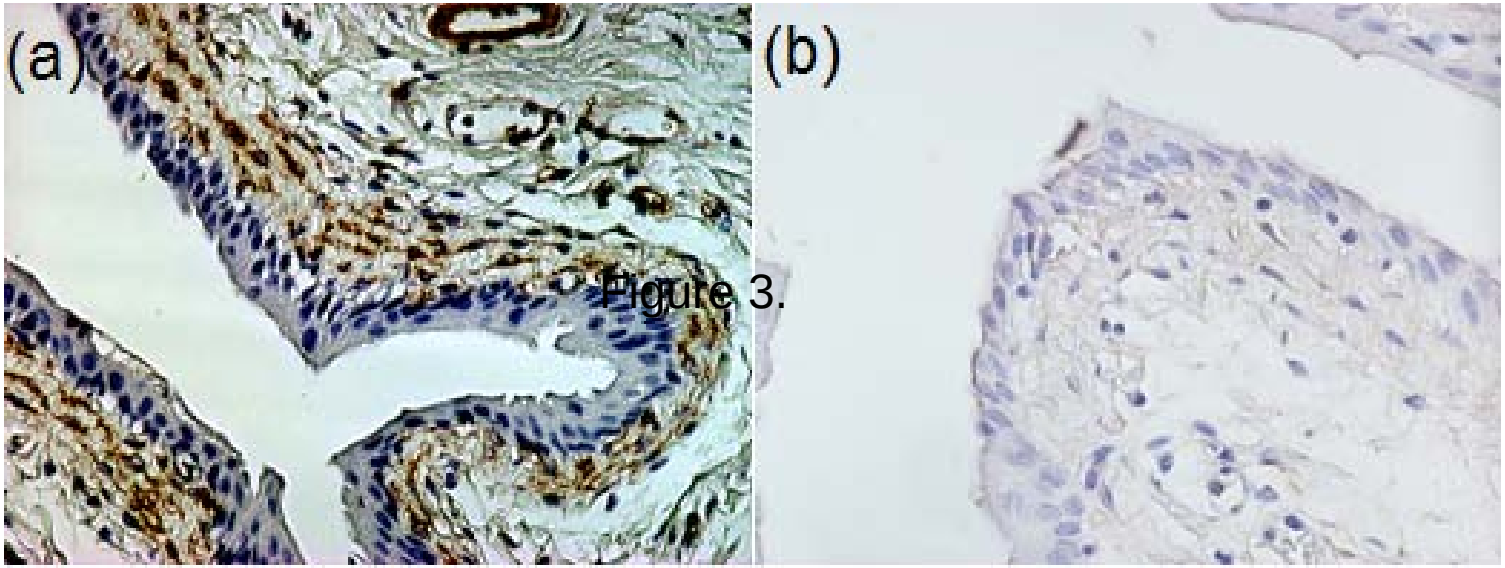

Figure 3.

Figure 3.

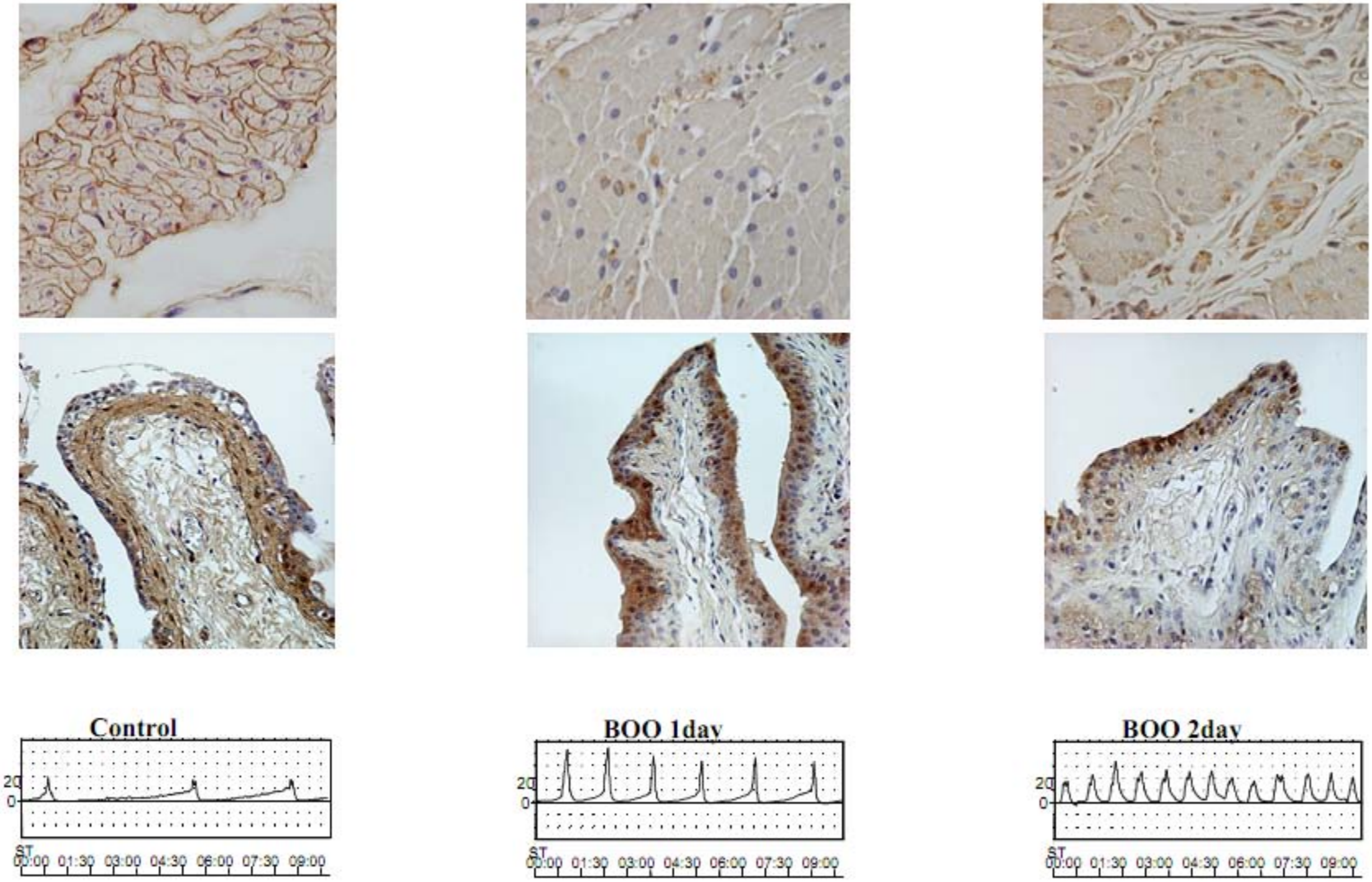
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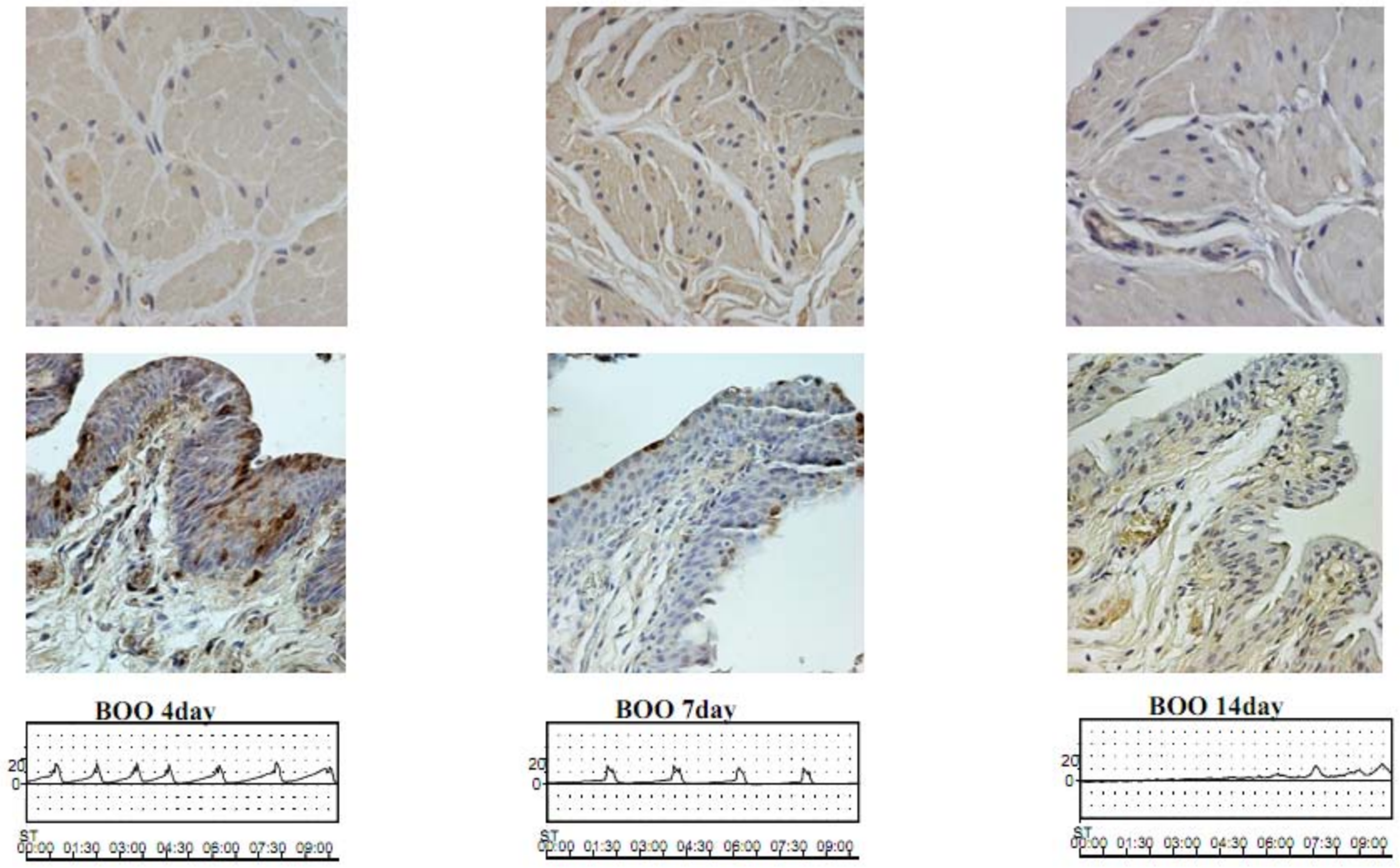
Figure 3.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