The parasympathetic nerves regulate colonic motility and defecation. The vagal nerve controls the right colon and the pelvic nerve permeates the left colon and rectum via the rectal branches of the pelvic plexus (RBPP). This investigation aimed to measure the functional changes of the colon and rectum after RBPP-transection for over six months.

RBPP-transection was performed in 15 dogs. Five dogs each were sacrificed immediately, one month, and six months after RBPP-transection. The stool condition, colorectal transit, defecation reflex, colorectal response to electrical stimulation, and pathological degeneration was investigated prior to, one month after, and six months after RBPP-transection.

Four of the 5 dogs observed had loose stool one month after RBPP-transection, and one of the 3 had recovered six months later. Half transit time (HTT) at transverse colon got longer in six of the 8 in one month. Six months later, HTT got shorter in three of the 4 than that of one month. Defecation reflex was not observed one month after RBPP-transection, but noted in two of the 5 six months later.

These results may suggest that vagal nerve compensates for the oral site of the left colon after denervation of the pelvic nerve which is originally distributed.

Key words: Pelvic nerve, Rectal branches of pelvic plexus, Colonic motility, Defecation

Introduction

Several studies have been conducted showing that some kinds of transit abnormalities are present in patients with constipation. Transit studies using radiopaque markers or scintigraphy revealed two patterns of the transit disorders: The colonic inertia in the left side colon and motility disorder in the right side colon.

Anterior resection surgery for rectal cancer (AR) causes urinary disturbances and sexual dysfunction, which is secondary to autonomic nerve injury. The relationship between autonomic nerve injury and these complications has been reported and studied in detail by many investigators. Many functional disorders in colonic motility and defecation have been observed after AR. Frequent and irregular evacuation, sensory disorders, and soiling are the major disorders. Furthermore, disturbance of stool passage in the colon and rectum was observed after AR. Stool is retained in the left side of the colon, which is proximal to the anastomosis. Meanwhile, it has been demonstrated that patients with a history of hysterectomy also suffer from defecation disturbances.

These functional disorders were previously thought to be caused by injury to the pelvic nerve. The quantitative changes in colorectal function after transection of the rectal branches of pelvic plexus (RBPP) have not been intensively studied. The purpose of this investi-
gation was to measure the functional changes of the colon and rectum after RBPP-transection for over 6 months.

Since the canine colon resembles the human colon, adult mongrel dogs were used in this study. We severed the RBPP bilaterally and performed physiological and pathological examinations regarding defecation and colonic motility. The pelvic plexus of the dog is composed of hypogastric nerve and pelvic nerve\(^2,3,4,13\). Prior to this investigation, we have confirmed that no response was noted on the colon and rectum with electrical stimulation of the hypogastric nerve. On the contrary, the colon and rectum had response with the pelvic nerve stimulation, and almost the same response was noted with RBPP stimulation. It is known that the right colon is under the control of the vagal nerve, and the pelvic nerve sends signals to the left colon and rectum via the RBPP. Therefore, in our study, RBPP-transection means denervation of the left side of the colon in the parasympathetic nerves system.

Materials and Methods

Animals

We used 15 adult mongrel dogs weighing 15 to 20 kg. All animals were handled in accordance to policies of the Animal Research Center of our University and according to the institutional guidelines for the care of laboratory animals. All the dogs had the rectal branches of the pelvic plexus (RBPP) severed (RBPP-transection). Prior to RBPP-transection, we observed the condition of defecation and evaluated the colorectal transit time. The RBPP was severed bilaterally after opening the abdominal cavity. Electrical stimulation to the RBPP and the colonic nerve (CN) was performed. The defecation reflex and colorectal responses were then observed. Then dogs were randomized into three groups. The immediate-group, 1M-group, and 6M-group. Five dogs were allotted to each group (Fig.1). The dogs in the immediate-group were sacrificed immediately after these procedures. Their colons were resected and kept in neutral buffered formalin. These were used as controls for the microscopic findings of the myenteric plexus. The remaining 10 dogs were housed and followed after the RBPP-transection. 5 dogs in the 1M-group had the condition of defecation and transit time examined one month after the RBPP-transection. Their abdomens were opened. The defecation reflex and colorectal responses to stimulation were observed. The dogs were then sacrificed and their colons were resected for microscopic observation. 5 dogs in the 6M-group had the condition of defecation and colorectal transit time observed at one and six months after RBPP-transection. Six months later, their abdomens were opened and the same examinations were performed as in the 1M-group and sacrificed.

Canine Autonomic Nervous System and Demarcations of Colon and Rectum

Fig. 2 is a schema of the anatomy of the canine colon and rectum\(^2,3,4,13\). The caudal mesenteric plexus (CMP) is composed of the intermesenteric plexus and lumbar splanchnic nerves (LSN). The colonic nerve (CN) is diverted from the CMP along the caudal mesenteric artery (inferior mesenteric artery in

![Fig. 1. The schedule of this study.](image-url)
humans). The CN is distributed on the left side of the colon and the rectum. Bilateral hypogastric nerves (HN) are diverted from the CMP and constitute the pelvic plexus (PP) with pelvic nerve (PN). The rectal branches of the pelvic plexus (RBPP) are distributed into the rectum.

Then we identified the following four sites: ICV-point, T-point, D-point, and R-point.

**Condition of defecation and colorectal transit time**

Prior to surgery, all dogs defecated and we observed the condition of the defecation (Fig. 1). To calculate the transit time, three types of radiopaque markers (SITZMARKS, Konsyl Pharmaceuticals, Inc., USA) were used. Twenty radiopaque markers were mixed with a portion of meat that contained a small amount of barium sulfate. The dogs each ingested 20 markers at the following three time periods before abdominal X-ray imaging was performed: 11, 14, and 17 hours. A different type of marker was used for each time period. Under anesthesia with ketamine hydrochloride, abdominal radiographs were taken from two projections. We then counted each type of marker in the following segments: oral to ICV-point, between ICV-point and T-point, between T-point and R-point, between R-point and anus, and defecated (Fig. 3). From the location of each marker, the half transit time (HTT) was calculated, which is the time when 10 markers passed the ICV-point, T-point, R-point, and anus. The times from the ingestion of the markers to taking the photographs were defined as independent variables and the numbers of markers that passed the point were dependent variables. Regression analyses were used to relate the times to the numbers with linear models. From the straight line, the time when 10 markers passed the point was calculated as the half transit time (Fig. 4).

**The RBPP severing operation**

The operation was performed under general anesthesia using ketamine hydrochloride (10 mg/kg body weight) and sodium pentobarbital (5 mg/kg body weight). A median abdominal incision was made and the abdominal cavity was opened. Bilateral RBPP and the CN were easily identified and exposed. Strain gauge force transducers (Starmedical F-12IS) were then sutured along the longitudinal axis on the surface of the T-point, D-point, and R-point (Fig. 2). A manometer, a pressure sensitive rubber balloon, was inserted into the anal canal. The RBPP was then severed bilaterally. After all physiological examinations, we removed the transducers and manometer. The dogs in the immediate-group were sacrificed. We returned the bowel and closed the wound in the dogs in the 1M- and 6M-group. Dogs recovered from anesthesia and were housed. The dogs in 1M-group underwent abdominal surgery again one month after RBPP-transection. The examinations were carried out, and the dogs were then
sacrificed. In 6M-group dogs, the same examinations were performed six months after the RBPP-transection. **Defecation Reflex and Colorectal responses by electrical stimulation**

During the operation, we inserted a manometer into the rectum and infused water at room temperature into the balloon. The rate of water infusion was 3-5
ml/second (rapid) and 0.5 ml/second (slow). We then observed the defecation reflex caused by the volume expansion of the balloon (Fig. 1).

After defecation reflex observation, stimulus parameters of 8-10 V, 2 ms, and 10 Hz were provided by a stimulator (model DPS-06, Dayna Medical Systems, Tokyo, Japan) and applied to the distal end of the RBPP and CN with a pair of wire electrodes. We used the same stimulus parameters proven to be valid for colonic response by Sato and Kihara. The colorectal responses were recorded through the strain gauge force transducers and manometer. Each transducer and manometer was connected to a pressure amplifier and pen recorder.

Sacrifice of the dogs and Microscopic features of the myenteric plexus

After sacrifice of all the dogs, we resected the ascending colon, transverse colon, and the rectum about 2 cm along the transverse axis. Then specimens were fixed in neutral buffered formalin (10%, pH 7.4) for 24 to 48 hours. After embedding in paraffin, 3 μm thick sections were processed. The sections were then deparaffinized and stained with hematoxylin and eosin (HE). For immunohistochemical examination, monoclonal mouse anti-human Neuron Specific Enolase (Anti-NSE; DAKO U7026), rabbit anti-cow S100 (Anti-S100; DAKO U029), and monoclonal mouse anti-human neurofilament protein (Anti-Neurofilament; DAKO-NF, 2F11) were used. Sections were covered with Anti-NSE and anti-S100 for 60 minutes at room temperature, and with anti-Neurofilament for 12 hours at 4°C. Hematoxylin was used as a counterstain. The myenteric plexus was observed, and the number of ganglion cells with nuclei in an ×200 field was counted. The average number of ganglion cells in 5 fields was calculated. The immediate-group was used as the control. The Mann-Whitney U test was used to determine any significant differences between the immediate-group versus the 1M-group, and the immediate-group versus the 6M-group in the ascending colon, transverse colon, and rectum.

Results

Conditions of defecation and colorectal transit time

Prior to RBPP-transection, all the dogs evacuated a large amount of stool at one time. None of them had diarrhea or constipation. One month after RBPP-transection, we observed the defecation condition in 5 of the 10 dogs in 1M- and 6M-group. Among the 5 dogs, 4 dogs had loose stool. Six months after RBPP-transection, we observed the defecation condition in 3 of the 5 dogs in 6M-group. Among the 3 dogs, only one had recovered in the stool condition, however the other two had loose stool. In these observations, not all of the dogs were observed to evacuate the stool.

The half transit time (HTT) is shown in Fig. 5. In 4 dogs, the HTT at the ICV-point got longer one month after the RBPP-transection. However, no trend was observed in 6 months. At the T-point, we can observe the change of HTT in eight dogs at one month after RBPP-transection. As for the 6 of the eight dogs, the HTT got longer. Six months after RBPP-transection, the HTT got shorter than those in one month in 3 of the four dogs. At R-point, in 6 of the eight dogs the HTT got longer one month after RBPP-transection. And in 3 of the four dogs, it got shorter six months later. At the anus, in 4 of the six dogs, the HTT got longer one month after RBPP-transection. But no trend was observed in six months.

Defecation reflex and colorectal response by electrical stimulation

Before RBPP-transection, all dogs evacuated the balloon in their rectum only when the balloon was rapidly (3-5 ml/second) expanded. At the time the balloon was evacuated, the left side of the colon was drawn into the pelvis, and the balloon and stool were evacuated at once. This movement was recorded with transducers on the colon and rectum (Fig. 6). Almost the same movement was recorded in all of the dogs. When the volume was slowly (0.5 ml/second) expanded, the balloon was not evacuated. One month after RBPP-transection in the 1M-group dogs, the balloon was evacuated when its volume was rapidly expanded, but the left side of the colon was not drawn into the pelvis. Six months after RBPP-transection, the week movement was noted in 2 of the five dogs. In the remaining three dogs, this movement was not observed.

An example of the response of the colon and rectum to the electrical stimulation, which was recorded through the strain gauge force transducers and manometer, is shown in Fig. 7. The colorectal response ratio is the number of response-positive dogs / the number of electrically stimulated dogs. The colorectal response ratios to electrical stimulation to the CN and RBPP immediately, one month, and six months after RBPP-transection are shown in Table 1.
months after RBPP-transection are shown in Fig. 8. A wide segment of the left side of the colon reacted to RBPP stimulation immediately after RBPP-transection. In both the 1M- and 6M-group, no response was noted at the T-point and D-point. The response ratio at the R-point and anus was 0.2. With stimulation to the CN immediately after RBPP-transection, one half of the dogs had responses at the T-point and many had a response at the D-point, R-point, and anal canal. With stimulation to the CN one and six months after RBPP-transection, the response ratio at each point was still high.

Microscopic findings of the myenteric plexus
The number of ganglion cells in the ascending colon was $4.24 \pm 0.97$ (immediate-group, SD), $3.88 \pm 0.54$ (1M-group, SD), and $3.44 \pm 0.33$ (6M-group, SD). In the transverse colon, $3.48 \pm 0.61$ (immediate-group, SD), $4.36 \pm 1.15$ (1M-group, SD), and $3.36 \pm 1.67$ (6M-group, SD). In the rectum, $4.28 \pm 0.98$ (immediate-group), $3.56 \pm 0.55$ (1M-group, SD), and $2.48 \pm 0.23$ (6M-group, SD). The number of ganglion cells in the rec-
tum decreased in the 6M-group (p < 0.05). No statistically significant differences were observed in the ascending and transverse colon in both the 1M-group and 6M-group. No statistically significant difference was observed in the number of ganglion cells in the rectum in the 1M-group (Fig. 9). In the rectum of the 6M-group, some ganglion cells shrank in size. The cytoplasm became pale and homogenous. Also, focal hyperplasia of the plexus was observed in the rectum, and nerve fibers were observed as having a wavy structure (Fig. 10).
Prior to RBPP-transection, the response ratio to stimulation of the RBPP at the T-point and R-point was high, and both points were under the control of the RBPP. One month after RBPP-transection, the transit time at the T-point and R-point were delayed, and the response to the RBPP was not noted. Hence, the T-point and R-point were damaged in the parasympathetic systems by RBPP-transection. Six months later, HTT at T-point got shorter than one month in 3 of the four dogs. No response was noted by RBPP stimulation; however, the R-point had a response in 20% of the dogs (Fig. 5). The number of ganglion cells decreased in the rectum, but did not change in the transverse colon. These findings may suggest that the T-point may
have changed to the control of the vagal nerve in six months. That is, the oral site of the colon that is affected by the pelvic nerve may change to the control of the vagal nerve in six months after pelvic nerve denervation (Fig. 11).

The pressure sensation at the rectum is important for the defecation reflex. Rapid expansion of the rectum was found to be required for defecation reflex in our studies. Rectal mucosal stimulation induced activation of colorectal motility and relaxation of the anal sphincter. This response is chiefly due to the mucosal intrinsic reflex via the ganglion cells in the intramural plexus. One month after RBPP-transection, the balloon was evacuated when it was rapidly expanded, but the left side of the colon was not drawn into the pelvis. This is considered to be a local response, such as the recto-sphincter reflex. Since the extrinsic nerve was severed, the intramural plexus was considered to play a large role in this response. Six months later, the defecation reflex was recognized in 2 of the five dogs. These results might elucidate the improvement of the state of defecation in six months. However, the movement was weaker to the movement prior to RBPP-transection. So six months are not enough for the functional recovery of rectum and anus. As the response ratio got low and HTT delayed at the R-point after RBPP-transection, the RBPP can be considered to regulate and integrate each response, which results in defecation.

Reconstruction of the intramural nerve plexus after severing the extrinsic nerve has been reported. Bilateral RBPP of mongrel dogs were severed and the intramural plexus was observed. Oligogangliosis and hyperplasia of the nerve fibers in the myenteric plexus were recognized 2 weeks after the operation. Others have reported a decrease in acetyl choline esterase activity in the myenteric plexus 12 weeks after severing the extrinsic nerves. In our study, oligogangliosis in the rectum and structural changes of the neuron were observed. These changes may be explained with Wallerian degeneration and transneuronal degeneration. Focal hyperplasia of the plexus was observed and the nerve fibers extended had a wavy structure. This might suggest intrinsic neural network reconstruction in the left side of the colon.

Fig. 11. The distribution of parasympathetic nerve to the colon. The region affected by vagal nerve may get extended six months after pelvic nerve denervation.
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