Electrical neuromodulation at acupoint ST36 normalizes impaired colonic motility induced by rectal distension in dogs

Haifeng Jin,1,2,3* Jiemin Liu,1,2,4* Robert D. Foreman,2 Jiande D. Z. Chen,1,5,6 and Jieyun Yin1,6

1Veterans Research and Education Foundation, Veterans Affairs Medical Center, Oklahoma City, Oklahoma; 2Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; 3The First Affiliated Hospital of Zhejiang Chinese Medicine University, Hangzhou, China; 4Guizhou Provincial People’s Hospital, Guiyang, Guizhou, China; 5Ningbo Pace Translational Research Center, Beilun, Ningbo, China; and 6Division of Gastroenterology and Hepatology, Johns Hopkins Center for Neurogastroenterology, Baltimore, Maryland

Submitted 30 December 2014; accepted in final form 17 June 2015

Jin H, Liu J, Foreman RD, Chen JD, Yin J. Electrical neuromodulation at acupoint ST36 normalizes impaired colonic motility induced by rectal distension in dogs. Am J Physiol Gastrointest Liver Physiol 309: G368–G376, 2015. First published June 25, 2015; doi:10.1152/ajpgi.00467.2014.—Electroacupuncture (EA) has been shown to improve impaired gastric motility and slow waves in both humans and animals. However, its effects on colonic motility have rarely been investigated. The aim of this study was to investigate the effects and underlying mechanisms of EA on impaired colonic motility induced by rectal distension (RD) in dogs. Colon contractions and transit were measured in various sessions with and without EA in hound dogs chronically placed with a colonic cannula. Colonic contractile activity was assessed by motility index (MI). Autonomic functions were determined by the spectral analysis of the heart rate variability derived from the electrocardiogram. It was found 1) RD suppressed colonic motility by 40.5% (10.8 ± 0.9 with RD vs. 6.4 ± 0.8 at baseline, P < 0.002). EA at ST36 normalized colonic contractions suppressed by RD (12.9 ± 2.8, P < 0.002 vs. RD and P = 0.1 vs. control). 2) Administration of atropine blocked the ameliorating effect of EA on colon motility. 3) RD also delayed colonic transit (65.0 ± 2.0% with RD vs. 86.0 ± 1.9% without RD, P < 0.001) that was restored with EA (84.0 ± 1.9%, P = 0.178 vs. control). 4) EA increased vagal activity suppressed by RD (0.37 ± 0.07 with RD + EA vs. 0.09 ± 0.03 with RD without EA, P < 0.001). In conclusion, RD inhibits colonic contractions and delays colonic transit in dogs; EA at ST36 restores the RD-induced impairment in both colonic contraction and transit by enhancing vagal activity and mediated via the cholinergic pathway.

gastrointestinal motility; colonic motility; electroacupuncture; electrical stimulation; vagal pathway

THE FUNCTIONAL GASTROINTESTINAL disorders (FGIDs) are highly prevalent, accounting for 45–68% of gastrointestinal presentations to primary care facilities. It is estimated that 35 million individuals suffer from constipation in the United States (11). Irritable bowel syndrome (IBS) represents 29.8% (or 2.4–3.5 million) of all physician visits per annum in the United States (14). FGIDs cost healthcare industries hundreds of billion per year in United States (4, 11, 16). At least two of major FGIDs are related to dysfunction of colonic motility: chronic constipation and IBS. Colon dysmotility may cause symptoms, such as difficult defection, diarrheal, and bloating, and also some upper gastrointestinal symptoms such as upper abdominal discomfort or pain and vomiting (51, 61).

Rectal distension (RD), attributed to fecal stasis in the colon-rectum region, is known to induce gastrointestinal dysmotility and certain gastrointestinal symptoms due to inhibitory reflexes (9, 25). In experimental settings, RD induced by intrarectum balloon distention has been reported to impair gastric motility, such as gastric slow wave dysrhythmia, attenuated antral contractions, and delayed gastric emptying, as well as colonic motility (7, 8, 66).

Main methods for the treatment of chronic constipation and constipation dominant IBS (C-IBS) include diet, pharmacotherapy, and biofeedback training (10, 23, 48). For diet treatment, fibers have long been used for treating functional bowel disease symptoms; the fibers are one of the safest and most important therapies for constipation (37). Increased dietary fiber intakes has been reported to reduce constipation rates and result in significant cost saving (50). However, even when used carefully, fibers may exacerbate abdominal distension and constipation (15). Therefore, some nuances are necessary regarding timing, dose, and types, tailored to the need of individual patients. A meta-analysis on the effect of dietary fibers on constipation showed that dietary fiber intake could obviously increase stool frequency in patients with constipation but without obvious improvement in stool consistency, laxative use, or painful defecation (64).

For pharmacotherapy, bulk forming and osmotic laxatives are used by patients with mild constipation, but more evidence is needed to support the long-term use of them (24, 54). Biofeedback has been reported to be effective in treatment of constipation with paradoxical contractions of the rectum and anal sphincter (1, 48) but not for constipation with slow transit. Some overlap symptoms of those diseases are resistant to those treatments because of the weak effects of some drugs or the long-term history of diseases, and some of therapies are limited due to their side effects (11). Therefore, safe and effective methods for treatment of those diseases are deeply needed.

Electroacupuncture (EA) at ST36 (Zusanli), a modified method Traditional Chinese Medicine, has been shown to be effective in the treatment of various functional gastrointestinal diseases (13, 29, 65). In our previous study, we found EA at ST36 was able to accelerate gastric emptying; restore RD-induced impaired gastric motility in dogs, possibly by enhancing vagal activity (8, 44); and enhance intestinal contractions and glucagon-induced hypomotility in the fed state, and accelerated intestinal transit via the opioid and cholinergic pathways in dogs (53). However, to the best of our knowledge, little is
known whether EA at ST36 is able to improve impaired colon motility, such as slow transit and hypomotility of the colon.

Therefore, the aim of this study was to investigate effects and mechanisms of EA at ST36 on RD-induced impairment in colonic contractions and transit in dogs.

MATERIALS AND METHODS

Animal preparation. Five adult female hound dogs (3–4 yr, 22–25 kg) were used in this experiment. After an overnight fast, the dog was anesthetized with sodium thiopental at a dose of 5 mg/kg iv (Pentothal; Abbott Laboratories, North Chicago, IL) and maintained on IsoFlo (isoflurane 1.5%, inhalation anesthesia; Abbott Laboratories) in oxygen-nitrous oxide (1:1) carrier gases delivered from a ventilator following endotracheal intubation. Laparotomy was performed with the vital sign monitoring of mucosal color, pulse rate, and breathing rate. A colonic cannula was chronically and surgically placed in the anterior of the ascending colon ~5 cm distal to the cecum, as detailed in previous studies (7, 49). The cannula was used for the insertion of a manometric catheter into the colon for the assessment of colon contractions and the insertion of markers for the assessment of colon transit.

The female canine menstrual cycle usually lasts 1–3 wk and takes place only once or twice a year. During the cycle, a small amount of bleeding may be noticed (8). To avoid possible effects of menstrual cycle on colon motility, none of the experiments were performed during the menstrual period. The Animal Care and Use Committee of the Veterans Affairs Medical Center (Oklahoma City, OK) approved the protocol of this study.

Experimental protocol. The study was initiated after the dogs were completely recovered from the surgery (~2 wk after the surgery). The experiment was composed of six sessions in the postprandial state on separate days in a randomized order described as follows below (Fig. 1).

Session 1 (control) served as a control without any intervention. The recordings were made for three 30-min periods immediately after a standard test meal. Session 2 (RD) was designed for studying the effect of RD on colonic contractions and transit. RD was performed at 33 mmHg during the second 30-min period. Session 3 (RD + EA) was for investigating the effect of EA at bilateral ST36 on the RD-induced suppression of colonic contractions and transit. RD and EA were performed simultaneously during the second 30 min. Session 4 (RD + sham-EA) was a sham stimulation session in which RD and sham-EA (needles were inserted at ST36 without electrical stimulation) were performed during the second 30-min period. Sessions 5 and 6 were designed for determining the possible mechanism of EA involving the cholinergic pathway. In session 5, RD, EA at ST36, and intravenous infusion of atropine (0.05 mg/kg iv) (41) were given during the second 30-min period. Session 6 was the same as session 5 except that no EA was performed. Colon contractions were measured during the entire experimental session immediately after the meal.

The procedure for each session was as follows: after an overnight fast, one bottle of enema liquid (Fleet enema saline; C.B. Fleet, Lynchburg, VA) was given to each dog at 9:00 AM 1 h before the experiment. The experiment was performed in the laboratory with the animal standing on a table. During the study, the animal was minimally restrained: the dog was standing on the experimental table freely with its leash loosely attached to a fixed location at the same height to prevent the dog from jumping off the table accidentally (never happened in the study). To let the dogs accommodate to this experimental condition, they were trained to stand on the table for 3 h every day for 1 wk before the initiation of the study (8, 66).

Each session was initiated immediately after a solid test meal consisting of 375 g beef (Pedigree, Pedigree-Chopped Chicken). Colonic contractions, rectal tone, and electrocardiogram were simultaneously recorded in all these sessions. Animal behaviors were closely monitored and recorded during the experiment.

RD and measurement of rectal tone. A double-lumen catheter with a polyethylene balloon on its tip was inserted and positioned into the rectum with the proximal edge of the balloon 5 cm from the anal verge (34). The catheter was connected to an electronic barostat system (Distender Series IIR, Toronto, Canada) for RD

Fig. 1. Experiment protocol. Colon contractions were measured during the entire study immediately after the meal.
and the measurement of rectal tone. The pressure of RD was set at 33 mmHg, which the animals tolerated well without uncomfortable behaviors. The rectal tone was assessed by averaging the rectal volume during the last 10 min of RD; a higher volume was indicative of lower rectal tone; whereas a lower volume reflected higher rectal tone (34).

**Electroacupuncture.** Bilateral acupoints, ST36 were inserted with acupuncture needles for EA in this study. Sham-EA was performed by inserting needles at the ST36 without stimulation. A universal pulse generator (model DS8000; World Precision Instruments, Sarasota, FL) connected to the needles inserted into ST 36 was used to generate the electrical current for EA. The electrical stimuli consisted of pulse trains. The frequency of the pulse trains was 12 trains/min, the duration of each train was 2 s, and the pulse amplitude was 3–6 mA according on animal tolerance and behaviors. The frequency and width of pulses in each train were 25 Hz and 0.5 ms, respectively. The choice of this parameter set was based on our previous studies (8, 31, 45). EA at 25 Hz has been consistently and robustly shown to improve gastrointestinal motility. In a previous study, however, EA at 2 Hz was found to be more potent in soliciting cardiovascular responses than frequencies higher 2 Hz (68).

**Recording and analysis of colonic contraction.** The colonic cannula was opened after the solid test meal, and a manometric catheter was inserted into the colon through the cannula toward the direction of the rectum. An eight-channel water-perfused manometry system (Medkinetic, Ningbo, China) was used to record colonic contractions during the entire period of each session. After the recording, the colonic contractions were analyzed using a custom-made software (Medkinetic). Motility index (MI) of the colonic contractions was defined as the mean area under the curve of each contraction.

**Recording and analysis of colonic transit.** At the end of each study session, 20 polyvinyl ring markers (diameter of 5 mm and length of 2 mm) were placed into the ascending colon through the cannula. Colon transit was assessed by collecting and calculating the percentage of markers defecated at 24, 48, and 72 h after the insertion. During the subsequent 72 h, the animals were given regular food and water without any intervention.

**Recording and analysis of autonomic function.** The autonomic function was assessed from the spectral analysis of the heart rate variability (HRV) signal derived from the ECG described previously (8). The ECG was recorded during each session (see Fig. 2A). Custom-made software was used to detection R waves from the ECG signal, which resulted in R-R intervals (Fig. 2B). An HRV signal was obtained by interpolating and uniformly sampling the R-R interval signal (Fig. 2C). Finally, the power spectral analysis was performed on the HRV signal (Fig. 2D). The power in the low-frequency (LF) band (0.04–0.15 Hz) represents mainly sympathetic activity, and the power in the high-frequency (HF) band (0.15–0.50 Hz) represents vagal activity (8, 66).

**Statistical analysis.** Statistical analyses were performed using SigmaStat17.0 (SPSS, Chicago, IL). Data are reported as means ± SE. ANOVA was applied to investigate the difference among different sessions. Student’s t-test was used to investigate the difference between any two periods or two sessions. For multiple comparisons, the
Tukey’s method was used and $P < 0.05$ was considered statistically significant.

RESULTS

All dogs tolerated the procedures well. A pressure of 33 mmHg of RD maintained by the barostat system was the highest pressure the dogs could tolerate without adverse behaviors indicative of pain or discomfort.

Effects and mechanisms of EA on colonic contractions. RD inhibited colonic contractions. The MI was $10.8 \pm 0.9$ at baseline, reduced substantially to $6.4 \pm 0.8$ during RD ($P < 0.002$ vs. baseline) and $7.6 \pm 0.9$ ($P < 0.05$ vs. baseline) during the 30 min after termination of RD.

EA restored RD-induced inhibition on colonic contractions (Figs. 3 and 4). The MI during RD with EA at ST36 was $12.9 \pm 2.8$, which was significantly higher than that during RD without EA ($P < 0.002$) and comparable to the baseline value before RD ($P = 0.5$). However, this effect was not observed during sham-EA.

Atropine blocked the ameliorating effect of EA on colonic contractions. Administration of atropine did not alter MI (Fig. 4; $P = 0.4$, RD vs. RD + atropine). However, in the presence of atropine, EA was not able to enhance colonic contractions (Fig. 4; $P = 0.3$, RD + atropine vs. RD + atropine + EA).

Effects of EA on rectal tone. EA decreased rectal tone compared with the control session and sham-EA session. The intraballoon volume was higher during RD with EA than during RD without EA ($119.6 \pm 11.3$ ml with RD + EA vs. $90.9 \pm 9.7$ ml with RD without EA; $P < 0.04$). The effect of EA on rectal balloon volume was blocked by atropine ($84.4 \pm 5.3$ ml with RD + atropine + EA; $P = 0.3$ vs. RD only); atropine did not alter intraballoon volume (Fig. 6).

Autonomic mechanisms of EA. RD with a pressure of 33 mmHg decreased vagal activity (HF) from $0.31 \pm 0.03$ at baseline to $0.09 \pm 0.03$ ($P < 0.05$) and increased sympathetic activity from $0.70 \pm 0.03$ at baseline to $0.91 \pm 0.03$ ($P < 0.05$). EA normalized vagal activity that was decreased by RD ($P < 0.01$ vs. RD; $P = 0.5$ vs. baseline before RD; Fig. 7A). EA decreased sympathetic activity (Fig. 7B; RD + EA vs. RD, $P < 0.01$). The effects of EA on the autonomic functions were blocked by atropine (Fig. 7).

DISCUSSION

This current study demonstrated that RD inhibited colonic contractions and transit in dogs; EA at ST36 restored the impaired colonic contraction and transit induced by RD by enhancing vagal activity and mediated via the cholinergic pathway.

RD has been commonly used to establish a temporary model of impaired gastrointestinal motility in the research of gastrointestinal motility, such as function dyspepsia, constipation, and constipation-dominant IBS. In previous studies, RD was reported to induce upper and lower gastrointestinal symptoms (2, 32); inhibit gastric tone in a volume-dependent manner and impair gastric accommodation (28); impair gastric myoelectrical activity, which resulted in delaying gastric emptying and gastric dysrhythmia (38, 47); and inhibit small intestinal motility in humans and animals (25, 47). However, there is little information about the effects of RD on colonic contractions and colonic transit. This present study showed that RD at 33 mmHg significantly inhibited colonic motility, including reduced colonic contractions and delayed colonic transit in dogs. In this study, we performed RD after the meal, because of the following reasons: 1) the meal made the experimental condition consistent among different sessions; without the test meal, the colon contractile activity in different sessions might vary substantially; 2) the meal enhanced colonic motility and thus made it possible to observe a substantial inhibitory effect of RD on colonic motility.
RD; and 3) consistent inhibitory effects of RD on gastrointestinal motility have been observed after a meal in a number of our previous studies (8, 49, 66).

For the treatment of gastrointestinal motility disorders, acupuncture and EA have been accepted by more and more patients in treatment of functional gastrointestinal diseases because of their efficacy and acceptable safety profile (rare side effects) compared with pharmacotherapy (65). In our laboratory, EA at ST36 was found to accelerate gastric emptying and restore impaired gastric accommodation in dogs (44, 45, 66); EA at ST36 was able to improve upper and lower abdominal symptoms and impaired gastric slow waves induced by RD mediated via the vagal pathway (32); EA at ST36 improved glucagon-induced hypomotility in the fed state and accelerated intestinal transit via the opioid and cholinergic pathways in dogs (53). In colon, EA at ST36 was reported to increase contractility of the distal colon via the cholinergic pathway in conscious rats (36) and increase colonic transit mediated via the sacral parasympathetic efferent pathway in rats (22). While these previous studies have shown the enhancement of vagal activity with EA, EA and direct vagal nerve stimulation are two totally different methods although they may have some similar effects and mechanisms (18, 42). EA is noninvasive and has excellent safety profile. Whereas direct vagal nerve stimulation is invasive and may have some unknown sides effects that may prevent its clinical applica-

**Fig. 4.** Colonic motility index at baseline and during different interventions RD significantly decreased colonic motility index compared with baseline (*P < 0.002 vs. baseline) and EA but not sham-EA normalized RD-induced impairment of colonic motility (#P < 0.002 vs. RD without EA). Atropine blocked the effect of EA on colonic motility.

**Fig. 5.** Colon transit assessed by the defecated markers in 24 h. RD significantly decreased colonic transit compared with the baseline condition without any intervention (*P < 0.01 vs. baseline). EA but not sham-EA normalized impaired colonic transit induced by RD (#P < 0.01 vs. RD or RD + sham-EA; P = 0.796 vs. baseline). The effect of EA on RD-induced impairment of colonic transit was blocked by atropine; no difference between the RD + atropine session and RD + atropine + EA session.
previous studies, EA was performed bilaterally and therefore the same method was used in this study.

Previously, we studied the effects of EA on RD-induced dysmotility of the stomach. In this study, we investigated the effect of EA on RD-induced hypomotility of the colon. In a study of gastric motility, we found that electroacupuncture at ST 36 could improve impaired gastric motility and slow waves induced by RD in dogs (8) and restore impaired gastric accommodation in vagotomized dogs (44). Whereas the findings of this study suggested potential applications of EA for the treatment of slow-transit constipation and constipation-predominant IBS. In the present study, we found EA for 30 min could restore the impaired colonic contractions induced by RD in the postprandial state. The reason we just performed EA for 30 min was that in our previous study we found the prokinetic effect of EA with a duration of 30 min was similar to that of EA with a duration of 75 min on delayed gastric emptying in dogs, suggesting that prolonged EA might not be necessary (66). For stimulation parameters, we used the parameters optimized in our previous studies: 25 Hz, 2 s on, 3 s off, 0.5 ms; EA at ST36 with these parameters has been reported to increase gastric motility in rats (21, 67) and accelerate gastric emptying and enhance antral contractions in dogs (8, 45, 66).

Concurrent with the acute effect on colon contractions, a brief EA at ST36 was found to exert a prolonged effect on colon transit in the present study. Under normal conditions without any intervention, 86% of inserted markers were defecated in 24 h and all defecated in 48 h. RD for 30 min decreased the colon transit 41%, and this was normalized by EA at ST36 for 30 min. These findings suggest that both RD and EA have sustained and prolong effects on colon transit.

The utilization of the barostat device allowed us to perform RD at a fixed and constant pressure; meanwhile, it allowed us to assess rectal tone by measuring the intraballoon volume. Rectal tone has a close relationship with rectal pressure and relaxation of rectal muscle (27). The rectal pressure plays an important role in defecation, while rectal tone is important for rectal accommodation. In the stomach, EA at ST36 has been consistently reported to exert a prokinetic effect on gastric motility; meanwhile, it was also shown to reduce gastric tone and improve gastric accommodation. This is critically important as certain prokinetics, such as erythromycin, improve gastric motility but impair gastric accommodation by increasing gastric tone; as a result, these kinds of prokinetics are not effective in treating overall symptoms resulting from gastric dysmotility. Similar to its effects on gastric motility, EA at ST36 also showed an inhibitory effect on rectal tone while enhancing colonic contractions and transit. This finding suggests that EA at ST36 may reduce symptoms related to RD resulting from accumulation of feces.

Mechanistically, we found that RD substantially decreased vagal activity by 72% and significantly increased the sympathetic activity by 30%. The most common explanation on possible mechanisms of RD on colonic motor motility is that RD activates afferent mechanosensory fibers that project to the prevertebral ganglia integrated with central synaptic input and then conveyed to the superior mesenteric and celiac ganglia via intermesenteric nerves. The afferent input activates sympathetic neurons in the prevertebral ganglia and inhibits motor activity in the colonic segment proximal to the site of distension (20, 26).

EA at ST36 restored the RD-induced impaired colonic contraction and colonic transit by normalizing the sympathovagal balance that was impaired by RD. Location-specific effects of EA on autonomic function have been reported in the literature. In this study, EA at ST36 enhanced vagal activity and reduced sympathovagal balance (LF/HF). Whereas in a previous study (57), EA at PC6 was shown to modulate gastric distention-induced reflex sympathoinhibition and vagal excitation in rats. This opposite effect could be attributed to the use of a different stimulation location (PC6 vs. ST36) and/or different experimental conditions (sympathoinhibition and vagal activation vs. sympathoactivation and vagal inhibition in this study). In another study, however, clear location-specific effects were elucidated (21): in rats, EA at ST36 increased gastric motility and decreased the sympathovagal ratio, whereas EA at PC6 inhibited gastric motility and increased the sympathovagal ratio. Meanwhile, the normalizing effect of EA on colonic motility was found to be blocked by atropine as shown in Fig. 7. These findings suggest the involvement of the cholinergic pathway. However, there is a limitation in the use of atropine because atropine can cross the blood brain barrier.
and influence central cholinergic pathway and acetylcholine was reported to participate in central neural cardiovascular actions of EA (28a).

While the autonomic mechanism involved in the ameliorating effect of EA on RD-induced colon hypomotility was implicated, the central action of EA was not investigated in this present study. However, a number of previous studies have shown EA has both spinal and supraspinal actions with respect to its effects on autonomic outflow. Longhurst and colleagues (12, 58, 59) reported that during and after EA stimulation, nociceptin, γ-aminobutyric acid (GABA), and opioids, including endorphins, and enkephalins, acting through μ- and δ-opioid receptors in the rostral ventrolateral medulla (rVLM), inhibit sympathetic outflow and the resulting cardiovascular sympathoexcitatory response. Furthermore, it was reported that low frequency EA could release endocannabinoids and activate presynaptic CB1 receptors to inhibit GABA release in the ventrolateral periaqueductal gray (vIPAG) resulting in disinhibiting vIPAG cells to modulate the activity of rVLM neurons to attenuate the sympathoexcitatory reflex responses (60).

**Perspectives and Significance**

The findings of the present study suggest the potential of EA for the treatment for patients with gastrointestinal symptoms related to weak colonic contraction and slow colonic transit, such as constipation, constipation-dominant IBS or other colorectal disorders. Clinical studies are warranted to
explore such a therapeutic role of EA in the treatment of gastrointestinal symptoms related to impaired colonic motility.

In conclusion, EA at ST36 restores impaired colonic contractions and transit induced by RD by enhancing vagal activity mediated via the cholinergic pathway. EA may have a therapeutic potential for functional gastrointestinal disorders related to slow colonic transit.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: H.J., R.D.F., and J.Y. conception and design of research; H.J. and L.J. performed experiments; H.J. interpreted results of experiments; H.J. prepared figures; H.J. drafted manuscript; H.J., J.D.C., and J.Y. edited and revised manuscript.

REFERENCES

40. McNeerany TA, Sallam HS, Hunnicutt SE, Doshi D, Chen JD. Prolonged treatment with transcutaneous electrical nerve stimulation (TENS) modulates neuro-gastric motility and plasma levels of vasoactive intestinal...
Electroacupuncture on RD-Induced Impaired Colonic Motility


Ouyang H, Xing J, Chen JD. 43.

Noguchi E. 41.

Niu WX, He GD, Liu H, Qin XY. 40.

G376 Electroacupuncture on RD-Induced Impaired Colonic Motility

47.

Qian LW, Orr WC, Chen JD. 46.

Qian L, Lin X, Chen JD. 45.

Sallam HS, Chen JD. 49.

Takahashi T. 55.

Sun Y, Song G, Yin J, Chen J, Chen JH, Song J, Chen JD. 53.


Tjen-Alooi SC, Li P, Longhurst JC. 57.

Tjen-Alooi SC, Li P, Longhurst JC. 58.


Tjen-Alooi SC, Li P, Longhurst JC. 60.


Tjen-Alooi SC, Li P, Longhurst JC. 63.

Tjen-Alooi SC, Li P, Longhurst JC. 64.


Tjen-Alooi SC, Li P, Longhurst JC. 68.

Tjen-Alooi SC, Li P, Longhurst JC. 69.


