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Swallowing Disorder and Inhibition of Cough Reflex Induced by Atropine Sulfate in Conscious Dogs

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Abstract. In this study, the effects of atropine sulfate (atropine) on swallowing and cough reflex were evaluated in the two experimental models in conscious dogs. To evaluate the effects of atropine on swallowing, 1 mL of marker (contrast medium) was injected into the pharynx under X-ray exposure to induce swallowing. Baclofen, used as a positive control, caused marker congestion in the upper esophagus. In our experimental model, atropine (0.02 and 0.1 mg /kg, i.v.) dose-dependently increased not only the number of marker congestions but also that of the swallows. In addition, atropine significantly shortened the onset of first swallowing. In the evaluation of atropine effects on electrically evoked cough reflex induced by two electrodes implanted into the trachea, atropine strongly inhibited the number of coughs at 0.01 or 0.05 mg/kg accompanied with 0.01 or 0.05 mg/kg per hour (i.v.), respectively. These findings indicate that atropine has the potential of causing aspiration pneumonia through induction of swallowing disorder and inhibition of the cough reflex.

Keywords: swallowing disorder, inhibition of cough reflex, anti-cholinergic agent, dog, aspiration pneumonia

Introduction

Although anti-cholinergic agents are widely used for the treatment of various disorders, these agents are known to cause swallowing disorder and inhibition of cough reflex in rats, guinea pigs, and humans (1 – 5). However, the effects of anti-cholinergics on swallowing and cough reflex have not been fully investigated in non-rodents. Since swallowing disorder and impaired cough reflex are often recognized in patients with aspiration pneumonia (6), it is believed that these two adverse effects, when caused by pharmaceutical agents, might be accompanied by the severe condition, aspiration pneumonia. It is therefore significant to exactly evaluate the effects of anti-chloinergic agents on swallowing and cough reflex in pre-clinical studies using conscious dogs. In this study, we use two experimental models to evaluate the effects of atropine, an anti-cholinergic agent, on swallowing and cough reflex in conscious dogs. In our experiments, a contrast medium, 60% barium sulfate, was injected into the pharynx under X-ray exposure to induce swallowing. X-ray images of canine swallowing were recorded and evaluated by videofluoroscopic analysis. Baclofen was used as a positive control that causes the swallowing disorder, since baclofen has been shown to inhibit the number of swallows in humans (7). In addition, baclofen is known to induce dysphagia as a side effect. Before evaluating the effect of atropine on swallowing, we confirmed that this drug inhibits swallowing in our experimental model. With regard to the evaluation of the effects of atropine on the cough reflex, cough reflex was first triggered in conscious dogs by electrical stimulation through two wires implanted into the trachea. In the two experimental models used in this study, the number of congestions of contrast medium in the upper esophagus and the number of coughs were evaluated as swallowing disorder and inhibition of cough reflex, respectively, before and after administration of atropine.

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Materials and Methods

Animals and housing conditions

Beagle dogs of either sex (Covance Research Products, Inc., Princeton, NW, USA) weighing 7 to 14 kg were used in this study. Before the experimental phase, the animals were housed under standard controlled environmental conditions at 21°C – 25°C and 40% – 80% humidity with a 12-h light/dark cycle, fed once per day, and given water ad libitum. The dogs were allowed to acclimate to laboratory conditions for at least 1 month before the beginning of experiments and were confirmed to have no abnormal clinical signs in a 2-week pre-experiment observation period. All experimental protocols were approved by the Animal Care and Use Committee of Environmental Health Science Laboratory in Sumitomo Chemical Co., Ltd., and the experiments were performed in accordance with the Guidelines for Animal Care and Use Research.

Measurement of canine swallowing under conscious states

Canine swallowing was measured according to the method of Pollard et al. (8) with some modifications. Conscious dogs were manually restrained in right lateral recumbency on a fluoroscopy table of an X-ray apparatus (Toshiba medical supply Co., Ltd., Tokyo). In this posture, the contrast medium, 60% barium sulfate, was slowly injected as a marker into the canine pharynx using a catheter (Nelaton Catheter, Fr.16, with two holes; Termo Corp., Tokyo). During the marker injection, X-ray was irradiated from the lateral side to allow appropriate insertion of the catheter into the pharynx. In the beginning, the volume of the marker was increased stepwise (0.2, 0.5, and 1 mL) to determine the appropriate amount that induces swallowing. Injection of each volume of the marker was repeated five times at intervals of 1 min. X-ray output was set at 62 kW, 5.0 mAs, and 0.03 s. X-ray images were collected as moving images into a computer system by means of a digital video recorder software (InterVideo WinDVR; InterVideo Japan, Inc., Tokyo) at a rate of 30 frames/s. Beforehand, all animals were trained to lie quietly when put under the above experimental environment. The X-ray power was set to its minimum to screen out swallowing of the contract medium. After completion of the experiment, no abnormality due to X-ray exposure was observed in any dogs. All X-ray experiments were carried out safely by technicians wearing appropriate clothing (lead apron, gloves, and goggles).

When the effects of pharmaceutical agents on swallowing were evaluated, the volume of the marker was set to 1 mL. First, 1 mL of the marker was manually injected into canine pharynx for about 5 s. Immediately after repetition of marker injection five times at intervals of 1 min, baclofen or atropine was intravenously administered to the dog via the cephalic vein. The doses of baclofen were 0.3 and 1.5 mg/kg and those of atropine were 0.02 and 0.1 mg/kg. Ten minutes later, the series of marker injections was repeated again.

Analysis of canine swallowing

After the X-ray experiment, the images obtained were divided into 30 frames/s, and analyzed frame by frame. When dogs swallowed the marker, the bolus of the marker moved from the pharynx to the upper esophagus as a black shadow in the X-ray images. As for the swallowing, a lean of the epiglottis to the upper esophagus followed by esophagus relaxation was identified. However, there were cases where the marker could not pass through but stopped for a while in the upper esophagus. The former and latter cases were defined as marker swallowing and the marker congestion in the upper esophagus, respectively. The numbers of marker swallows and marker congestions in the upper esophagus were counted and evaluated during the repeated five trials of marker injection. In this experimental system, the number of marker congestions was considered as an index for swallowing disorder. Moreover, the frame number of X-ray images from the start of marker injection into the pharynx to the lean of the epiglottis against the upper esophagus was counted and defined as the onset of first swallowing.

Measurement of canine cough reflex under conscious states

Cough reflex was triggered according to the method of Gallico et al. (9) with some modifications. Two electrodes were chronically implanted into canine trachea in advance. Under anesthesia with pentobarbital at 30 mg/kg, i.v., the cervical midline was opened. The trachea was then exposed and implanted with two stainless wires (OD 0.25 mm; Unique Medical Co., Ltd., Tokyo) as bipolar electrodes at the distance of 4 cm from the cricoid cartilage. The tip of each wire was kept penetrating the tracheal mucosa. The bodies of wires were then covered with a polypropylene tube to insulate and prevent disconnection. Furthermore, a silicon tube (ID of 1.0 mm; Kaneka Medix Corp., Osaka) was inserted and sutured into left jugular vein for drug intravenous administration. The free ends of the wires and tube were brought out through a skin incision between the scapulae and protected with a jacket. After recovery from surgery, electrical stimulation was applied by means of an electronic stimulator (Nihon Kohden Corp., Tokyo) via the two wires to evoke cough reflex in

conscious states. Throughout the study, the number of electrically evoked coughs was counted by the same trained observer. The electrical stimulation was settled in each individual dog to trigger the 8 – 15 coughs consistently. Actual electrical stimulation consisted of 10 stimulations with a square-wave pulse of 1 ms, 50 Hz, from 8 to 20 V for 1 s, at an interval of 5 s. When electrical stimulation was repeatedly applied every 15 min and a consistent number of coughs was observed consecutively twice, atropine at 0.01 or 0.05 mg/kg was first intravenously given to the dog as a bolus and then by intravenous infusion at 0.01 or 0.05 mg/kg per hour over 30 min, respectively. Atropine was given via the silicon tube inserted into the left jugular vein. Electrical stimulation was applied again at the end of the atropine infusion and the number of coughs was counted.

Analysis of canine cough reflex

Cough reflex was evaluated by calculating percentage of the number of coughs after the infusion of atropine versus the average number of coughs at 15 and 0 min before that.

Statistical analyses

All results are presented as the mean ± S.D. For evaluation of drug effects on swallowing, statistical analyses of data were performed with the *F* test followed by Student's *t*-test or the Aspin-Welch *t*-test between the number of marker swallows, the number of marker congestions, and the onset of first swallowing before drug administration and those after administration. When the probability value by the *F* test was more or less than 0.25, data were statistically analyzed by Student's *t*-test or Aspin-Welch *t*-test, respectively. For evaluation of drug effects on cough reflex, statistical analyses of data were performed with Dunnett's multiple test between the percentage of the number of coughs in the atropine-treated group and that in the vehicle-treated group. A probability value less than 0.05 was considered as statistically significant.

Drugs

Baclofen and atropine were purchased from Nacalai Tesque, Inc. (Kyoto) and Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA), respectively. Both drugs were dissolved in saline. Barium sulfate was purchased from Sakai Chemical Industry Co., Ltd. (Osaka), diluted with distilled water, and used at a concentration of 60% (W/V).

Results

Marker swallowing and congestion in the upper esophagus

As shown in Fig. 1, the marker first accumulated little by little in the canine pharynx in the marker injection (Fig. 1B). After accumulation of a certain volume of marker, a lean of the epiglottis to the upper esophagus was then suddenly induced (Fig. 1C), followed by relaxation of the upper esophagus (Fig. 1D). The events accompanied by marker movement toward the stomach were considered to be swallowing (Fig. 1: C – F). In some cases, the marker could not pass through to the stomach but stopped in the upper esophagus for many seconds (Fig. 2: D – F). This was considered as marker congestion in the upper esophagus and defined as an index for swallowing disorder.

Induction of swallowing by marker injection

To determine the appropriate amount of marker that induces swallowing, the volume of marker was increased stepwise (0.2, 0.5, and 1 mL). As a result, the number of marker swallows increased in a volume-dependent manner (Fig. 3). In particular, when the marker was injected into the canine pharynx at the volume of 1 mL, marker swallowing was markedly induced. Dogs hardly swallowed the marker at the volume of 0.2 mL. Therefore, for evaluation of the effects of test drugs on swallowing, the volume of the marker required to induce swallowing was taken as 1 mL.

Effects of the positive control, baclofen, on marker swallowing

Baclofen was used in this study as a positive control that causes swallowing disorder in animals and humans. Baclofen dose-dependently increased the number of marker congestions in the upper esophagus (Fig. 4) with statistical significance at 1.5 mg/kg. These results indicate that the experimental system used in this study is valid and appropriate for evaluation of swallowing disorder induced by pharmaceutical agents. Baclofen, at any of the doses used, did not affect the number of marker swallows or the onset of first swallowing (Figs. 5 and 6). In the treatment with baclofen at 1.5 mg/kg, 2 out of the 6 dogs were in the prone position.

Effects of atropine on marker swallowing

Atropine dose-dependently increased the number of marker congestions in the upper esophagus (Fig. 4) with statistical significance at 0.1 mg/kg. In addition, atropine significantly increased the number of marker swallows at 0.1 mg/kg (Fig. 5). Atropine also signifi-

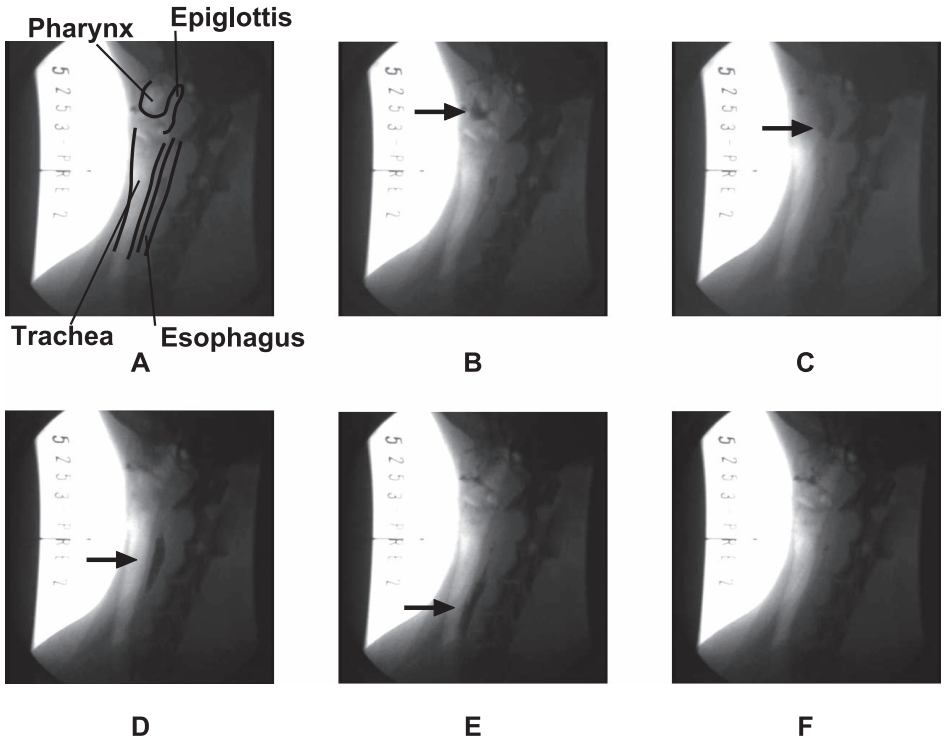

Fig. 1. Typical X-ray images of marker swallowing in conscious dogs. X-ray images are shown as lateral fluoroscopic pictures. Contrast medium, 60% barium sulfate (marker), is identified as a black bolus (arrows) in the pictures. A: The positions of the pharynx, epiglottis, esophagus, and trachea in an X-ray image are shown. B: A 1-mL volume of the marker was injected into the canine pharynx under the conscious state. C: Epiglottis leans to the upper esophagus. D, E, F: Marker passes through the esophagus to the stomach and disappears from the frame.

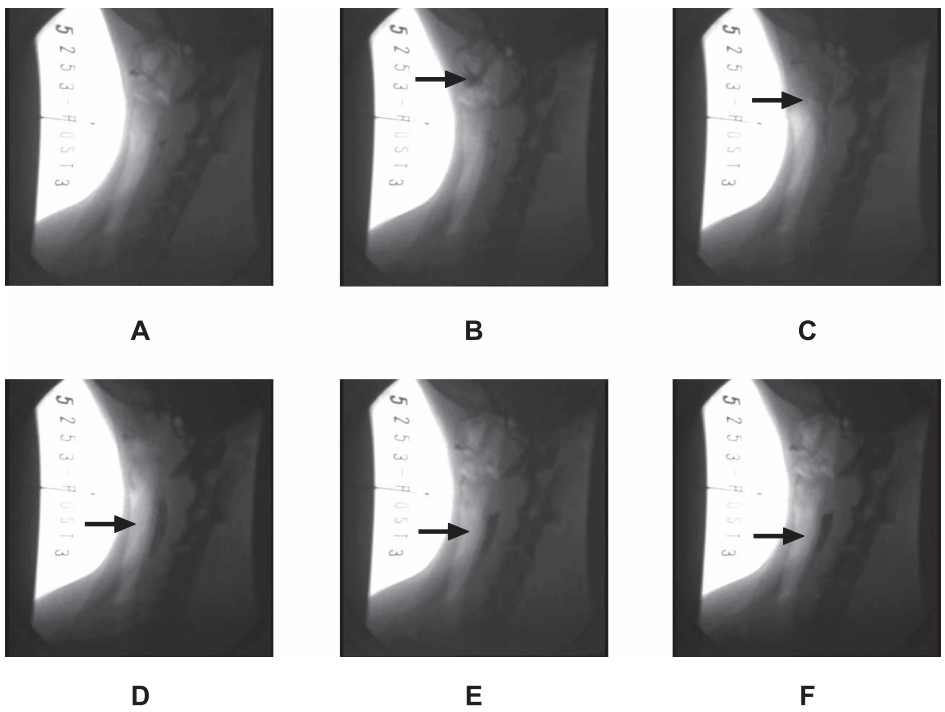

Fig. 2. Typical X-ray images of marker congestion in the upper esophagus. The experimental method is the same as that in Fig. 1. A, B, C: Images are the same as those in Fig. 1. D, E, F: Images are different from those in Fig. 1. The marker does not pass completely through the esophagus but stops in the upper esophagus.

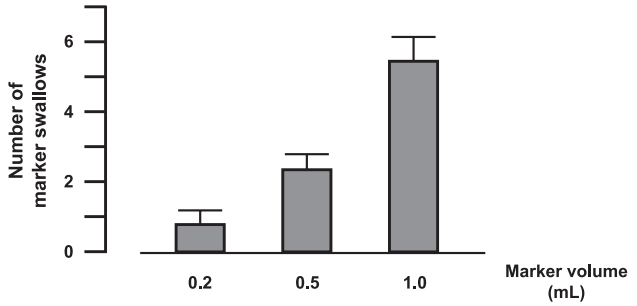

Fig. 3. Induction of swallowing in conscious dogs by injection of marker into the pharynx. The vertical index indicates the number of marker swallows in the five injections of the marker. Each column represents the mean ± S.D. of 6 dogs. Note that 1-ml volume of the marker drastically induced the marker swallowing.

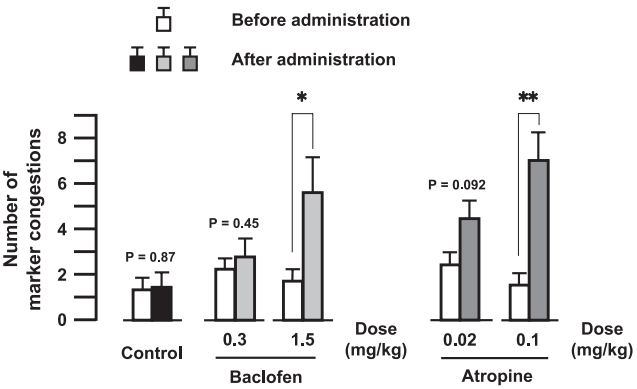

Fig. 4. Effects of baclofen and atropine on the number of marker congestions in the upper esophagus. The vertical index indicates the number of marker congestions in the upper esophagus in the five injections of the marker. White and colored columns show the number before and after drug administration, respectively. Each column represents the mean ± S.D. of 6 dogs. *P<0.05, **P<0.01: comparison between the number of marker congestions before and after treatment.

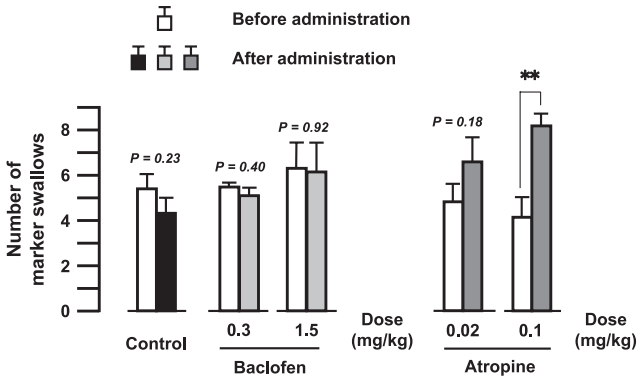

Fig. 5. Effects of baclofen and atropine on the number of marker swallows. The vertical index indicates the number of marker swallows in the five injections of the marker. White and colored columns show the number before and after drug administration, respectively. Each column represents the mean ± S.D. of 6 dogs. **P<0.01: comparison between the number of marker swallows before and after treatment.

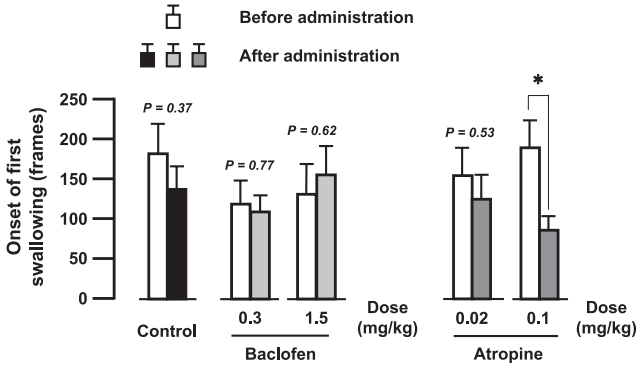

Fig. 6. Effects of baclofen and atropine on the onset of first swallowing. The vertical index indicates the average onset of first swallowing in the five injections of the marker. The number of X-ray images between the start of marker injection into the pharynx and the lean of the epiglottis to the upper esophagus was counted frame by frame and defined as the onset of first swallowing. The unit is frames, that is, the number of X-ray images. White and colored columns show the onset of first swallowing before and after drug administration, respectively. Each column represents the mean ± S.D. of 6 dogs. *P<0.05: comparison between the onset of first swallowing before and after treatment.

cantly shortened the onset of first swallowing at the dose of 0.1 mg/kg (Fig. 6). In the treatment with atropine, dogs tended to swallow a small volume of the marker before marker accumulated in the pharynx. After administration with atropine at 0.1 mg/kg, dry mouth and mydriasis were observed in all animals. Throughout the experimental period, induction of marker swallowing was not weakened by repeated marker injection into the pharynx.

Effects of atropine on electrically evoked cough reflex

In animals treated with the vehicle, a preset voltage of electrical stimulation triggered a consistent number of coughs (Fig. 7). Basal values of the number of coughs were 12.0 ± 1.8, 13.0 ± 0.0, and 11.6 ± 2.5 before administration of saline, atropine at 0.01 mg/kg + 0.01 mg/kg per hour, and atropine at 0.05 mg/kg + 0.05 mg /kg per hour, respectively. These basal values were not significantly different among the dosing groups. Atropine significantly decreased the number of coughs at 0.01 mg/kg + 0.01 mg/kg per hour, and atropine at 0.05 mg/kg + 0.05 mg/kg per hour in conscious dogs (Fig. 7). However, at the highest dose of atropine, dry mouth and mydriasis were observed in all animals. During the experimental period, the electrically evoked cough reflex was well reproducible.

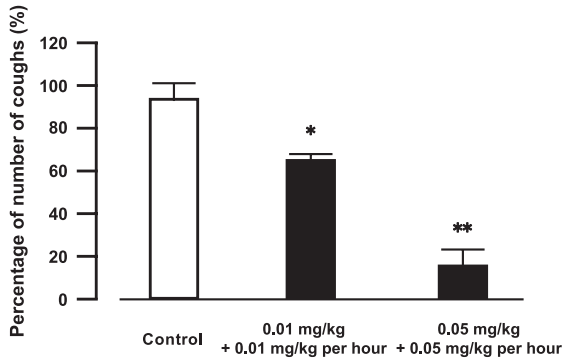

Fig. 7. Effects of atropine on electrically-evoked cough reflex. The vertical index indicates the percentage of the number of coughs after atropine treatment against the average of that before. Each column represents the mean ± S.D. of 4 dogs. *P<0.05 , **P<0.01: comparison with the control (vehicle).

Discussion

Using a contrast medium (marker) and X-ray analysis, we evaluated in this study the effects of atropine sulfate (atropine) on swallowing and cough reflex in conscious dogs. Our results show that atropine causes swallowing disorder and inhibits cough reflex. Indeed, in our experimental model, atropine increased not only the number of marker congestions in the upper esophagus but also the number of marker swallows. In addition, atropine at the dose of 0.1 mg/kg significantly shortened the onset of first swallowing. As for the effects of atropine on cough reflex, atropine significantly decreased the number of coughs at 0.01 mg/kg + 0.01 mg/kg per hour and 0.05 mg/kg + 0.05 mg/kg per hour.

It has been reported that water injection into the pharynx induces swallowing through activation of swallowing reflex in animals and humans (10 – 12). Accordingly, water injection is considered to be the most effective stimulus for elicitation of swallowing reflex through water-sensitive receptors in the pharyngolaryngeal region. In the dog model used in this study, swallowing reflex was elicited by injection of a contrast medium into the pharynx at the volume of 1 mL. This model might be more sensitive to induction of swallowing reflex since Lehmann et al. reported that the volume of water required to induce swallowing in dogs is about 4.4 mL (11). Since the dogs used in this study were kept in lateral recumbency, gravity might not work well on movement of the marker toward the stomach. In addition, it has been reported that the intra-esophageal pressure in this posture is about half that in the prone posture (13). Consequently, in our experimental system, marker injected into the pharynx might have caused congestion in the esophagus, making this model more sensitive to detection of the inhibitory effects of pharmaceutical agents on swallowing.

Baclofen is extensively prescribed as an antispastic agent (14). It has been shown that baclofen inhibits transient lower esophageal sphincter reflex not only in dogs but also in ferrets and human based on the activation of $GABA_B$ receptors (7, 15 – 17). Accordingly, it is believed that baclofen might induce dysphagia as a side effect. Therefore, we predicted that baclofen would decrease the number of marker swallows in our experimental system and used it as positive control. However, the finding that baclofen did not affect the number of marker swallows was unexpected and inconsistent with the results of a previous study showing that baclofen causes swallowing disorder (11). Further analyses demonstrated that the marker often congested in the upper esophagus of the animals given baclofen at 1.5 mg/kg. The significant increase in the number of marker congestions in the upper esophagus could support the swallowing disorder induced by baclofen in other animals and humans. These findings indicate that swallowing disorder induced by a pharmaceutical agent can be detected using our experimental model. Marker congestion induced by baclofen is believed to be due to a direct effect on an esophageal muscle or a peripheral muscle, such as the cricopharyngeus muscle, which is important for control of the swallowing response (18). The idea that baclofen affects swallowing reflex might be then excluded because baclofen did not affect the onset of first swallowing in this study.

Since our experimental system was considered to be valid for the evaluation of swallowing disorder induced by pharmaceutical agents, the effects of atropine on swallowing were evaluated in this system. It has been reported that atropine strongly inhibits motility in canine oesophageal sphincter (19, 20). In addition, acetylcholine is believed to be associated with swallowing (21, 22) and might stimulate contractile activity of the esophagus (23). Therefore, we anticipated that atropine would decrease the number of marker swallows in our experimental system. However, it actually did not. Atropine significantly increased not only the number of marker swallows but also that of marker congestions and shortened the onset of first swallowing. Though the animals were relieved from restraint 10 s after completion of marker injection, the marker congestion was observed for 10 s at maximum (300 frames). Some differences between the effects of atropine on swallowing and those of baclofen were observed. This is probably due to the different pharmacological profiles of these drugs. It is believed that atropine should activate the swallowing reflex to increase the number of marker swallows and shorten the onset of first swallowing. Animals given atropine tended to swallow a smaller

volume of marker more frequently before a certain volume of the marker accumulated in the pharynx. The threshold of marker volume for activation of swallowing reflex might be lowered by administration of atropine. On the contrary, the marker in the upper esophagus could hardly pass through the whole esophagus in animals given atropine. The increase in the number of marker congestions in the upper esophagus induced by atropine might be ascribed to dry mouth, collapse of esophageal peristalsis, inhibition of lower esophageal relaxation, or their combination (5). Considering that swallowing is a complex and highly coordinated neuromuscular process that involves the cholinergic nervous system, atropine might interfere with some functions in the nervous system to cause marker congestion. Swallowing is classified into three distinct phases: the oral phase, the pharyngeal phase, and the esophageal phase (24). Our results indicate that atropine activates the pharyngeal phase but inhibits the esophageal phase of the swallowing process.

The effects of atropine on cough reflex were also evaluated in this study. Cough reflex can be triggered by a various types of stimulations, including mechanical, chemical, and nervous stimulations (25 – 27). In the present study, the cough reflex was induced by electrical stimulation using two wires implanted into canine trachea. This methodology has the advantage of inducing a consistent cough response in conscious states. Before the effects of atropine on electrically evoked cough reflex were evaluated in our animal model, we confirmed that codeine phosphate, an antitussive drug, strongly inhibits electrically evoked cough reflex at 5 mg/kg, i.v. (data are not shown). Atropine significantly decreased the number of coughs at the end of its intravenous infusion. As the cholinergic nervous system is thought to be important in triggering the cough reflex, the inhibitory effect of atropine on cough might be associated with its relaxant effect on tracheal smooth muscle since muscarinic receptors are found in the smooth muscle of the airway (28, 29).

Our present approach related to the inhibitory effects of atropine on maker swallowing and cough reflex suggests the risk that atropine might induce aspiration pneumonia through swallowing disorder, inhibition of cough reflex, or their combination. Cough reflex and swallowing are closely associated with airway protective mechanism since they play a key role in preventing aspiration of foreign substances, internal secretion, or gastric contents into the trachea or lung. In animals with impaired cough reflex, swallowing disorder causes extensive accumulation of them in the upper esophagus and deteriorates aspiration of them into the trachea. In fact, aspiration of the marker into the trachea caused by an anti-cholinergic agent was also observed in our experimental model (Fig. 8). In addition, after completion of all the experiments related to the evaluation of the effects of test drugs on the cough reflex, the dogs treated with anti-cholinergic agents were sacrificed to scrutinize lung tissues. As a result, lung inflammation, with inflammatory scars localized near the tracheal branch, was observed in some dogs. It is therefore suggested that the anti-cholinergic agents cause aspiration of foreign substances into the trachea due to swallowing disorder and inhibition of the cough reflex and that these substances cause inflammation near the tracheal branch. Especially, it is believed that chronic administration of anti-cholinergic agents in elderly or bedridden patients might increase the risk of aspiration pneumonia. In the patients with aspiration pneumonia, the problem is thought to be due to not only dysphagia and inhibition of the cough response but also to vomiting, gastro-esophageal reflex disorder, or their combination (30 – 34). Therefore, we believe that our concomitant evaluation of swallowing disorder and inhibition of cough reflex has the advantage of predicting the serious risk that pharmaceutical agents cause aspiration pneumonia. In addition, the present two evaluations can be conducted without sacrifice of animals and before the generation of serious damage such as lung inflammation.

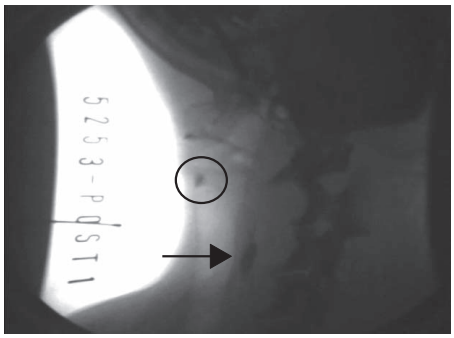

Fig. 8. Typical X-ray image of aspiration of marker into the trachea. As the arrow shows, a bolus exhibits marker congestion in the upper esophagus. Note that the marker introduced into the pharynx was detected not in the esophagus but in the trachea as bolus shown in the image by a circle shows.

In conclusion, we have shown in this study that atropine causes swallowing disorder and inhibition of cough reflex in conscious dogs. Although further investigation is required, our present results suggest that anti-cholinergic agents have the potential to cause aspiration pneumonia through swallowing disorder, inhibition of the cough reflex, or their combination.

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