Lab. Anim. Res. 2010: 26(3), 241-247

Laboratory Animal Research

http://submission.kalas.or.kr

Evaluation on Efficacy and Safety of Tribromoethanol and Tribromoethanol plus α_2-Adrenergic Agonists in Different Mouse Strains

Yoon Ju Cho†, Jae Won Lee, Jong In Kim, Young Ah Lee, Tae Yeon Kim and Jin Soo Han*

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University, Seoul, Korea

The present study was carried out to provide a guideline for injecting tribromoethanol (TBE) as the main anesthetic agent, while adjusting the doses of xylazine (X) and medetomidine (M) according to different strains of mice (male ICR, C57BL/6, and BALB/c). Seven intraperitoneal injection anesthesia protocols using TBE and mixtures of TBE and α_2–adrenergic agonists (TBE/X and TBE/M) were compared in terms of their efficacy and safety (anesthetic duration, death rate, and the development of pathological lesions of abdominal organs). All animals that were injected with a low dose of TBE (200 mg/kg) displayed clear signs of light anesthesia with a strong pedal withdrawal reflex. Despite the good anesthetic effect, a high dose of TBE (400 mg/kg) was not a suitable anesthetic for major surgery in all mouse strains because of the risk of pathologic changes in the abdominal organs, such as retention of the digestive tract, peritonitis, and fibrinoid adhesion. TBE200/X10 and TBE200/M0.5 (TBE, 200 mg/kg; X, 10 mg/kg; M, 0.5 mg/kg) appeared to be safe and provided satisfactory anesthesia in ICR mice. Finally, there were clear differences in anesthetic efficacy among ICR, C57BL/6, and BALB/c strains. TBE/M and TBE/X did not anesthetize BALB/c mice, and it anesthetized C57BL/6 mice for a short time. When administered with TBE/X and TBE/M maintained the sedation of ICR mice. We were able to establish different regimes for each strain (TBE200/X20 for C57BL/6, TBE300/X10 and TBE200/M1 for BALB/c). Our results showed that TBE/X and TBE/M could be recommended as an anesthetic mixture, with the dose appropriately adjusted according to mouse strain.

Key words: Anesthetic dose, strain difference, tribromoethanol, xylazine, medetomidine

Received 13 July 2010; Revised version received 29 July 2010; Accepted 31 August 2010

One of the fundamental components of veterinary medical care is the prevention or alleviation of pain from procedures and surgical protocols (National Research Council, 1996). The most appropriate anesthetic agent should be selected, in professional consideration of clinical and humane requirements, without compromising the scientific validity of the study (National Research Council, 1996; Bette *et al.*, 2004).

Tribromoethanol (TBE), a sort of alcohol, is one of the central nervous system depressor agents. TBE possibly exerts sedative and hypnotic effects through its interactions by enhancing $GABA_A$- and glycine-mediated synaptic inhibition (Ticku and Kulkarni, 1988; Mascia *et al.*, 1996). TBE has been used in anesthesia for laboratory mice because of its rapid induction to an adequate depth of surgical anesthesia and its relatively quick recovery period (Weiss and Zimmermann, 1999; Kiatchoosakun *et al.*, 2001). On the other hand, it has reportedly induced intestinal ileus, fibrous adhesions, muscle necrosis, serositis of several organs, and death in mice, especially at a high dose or with repeated use (Tarin and Sturdee, 1972; Green, 1975; Zeller *et al.*, 1998; Reid *et al.*, 1999; Gopalan *et al.*, 2005; Lieggi *et al.*, 2005a, 2005b).

Although studies are actively being undertaken to evaluate the effects and safety of TBE, 0.5 mL of 12.5 mg/mL TBE is used per animal in Korea without consideration of TBE's adverse effects. In addition, a wide range of TBE concentrations and doses has been reported in the literature (Table 1). Preliminary experiments conducted in our laboratory using TBE anesthesia on ICR mice yielded the following results:

†Current address: Department of Pet Science and Veterinary Nursing, Seojeong College University, Yangju, Korea

*Corresponding author: Jin Soo Han, Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Korea
Tel: +82-2-2049-6114
Fax: +82-2-3437-6114
E-mail: labvet@konkuk.ac.kr

Table 1. Guidelines for tribromoethanol anesthesia in several facilities or literatures in mice

Dose* (mg/kg)	References
125-250	• NIH Anesthesia/Analgesia formulary • Cornell Center for Animal Resources and Education, in Cornell University • Animal Care Facility in Laurentian University • Unit for Laboratory Animal Medicine in University of Michigan Medical School • Laboratory Animal Research Center in Samsung Biomedical Research Institute, Korea • Laboratory animal research center in Chungbuk National university, Korea
125-300	• (Flecknell, 2005) • (Fish *et al.*, 2008)
125-400	• Safety Services in University of California, Davis
225-240	• Research Animal Resources in University of Minnesota
250	• Guidelines in Duke University and Medical Center • Guidelines in University of Rochestar Medical Center • (Hedenqvist *et al.*, 2003)
250-500	• IACUC in University of California
300-600	• Animal Resource Center in Case Western Reserve University
310	• IACUC in University of Tennessee
360-800	• Guidelines in Baylor College of Medicine
400-600	• Office of the Campus Veterinarian in Washington State university
400-750	• Veterinary Services in Florida Atlantic University

*intraperitoneal injection

At all doses of TBE, anesthesia was induced within 1 min of administration. Anesthesia lasted for 10 and 33 min, on average, at 125 and 375 mg/kg, respectively. At 625 and 875 mg/kg, the subject mice perished within 3 min of administration. Although TBE is generally diluted to 1.25% to induce anesthesia in mice, 1.2 and 1.6 mL had to be administered at doses of 625 and 875 mg/kg, respectively, to subject mice whose average weight was 23 g. This was more than twice the recommended dose of 0.5 mL for abdominal injection (Hedenqvist *et al.*, 2003), leading us to believe that increased abdominal pressure was the cause of death. Accordingly, the experiment was conducted again by diluting TBE to 2.5%. Even at this concentration, between 0.6 and 0.9 mL, which exceeded the recommended dose, had to be administered to subject mice whose average weight was 23 g. The subject mice died within 7.5 min of TBE administration. As the preliminary experimental results indicate, laboratory animals dying due solely to anesthesia can cause major problems in conducting experiments. Experimental results can also be significantly affected by the adverse effects that manifest several weeks later.

In an attempt to reduce the side effects of TBE, Gopalan *et al.* (2005) have used a combination of a lower dose of TBE and medetomidine in SD rats. It is known that the duration of TBE anesthesia in mice varies considerably with mouse strain (Fish *et al.*, 2008). However, little is known about the variability of the anesthetic effect of combining TBE and α_2-adrenergic agonists (xylazine and medetomidine). The present study focused on the different anesthetic effect on each mouse strain (ICR, C57BL/6, BALB/c), and on mixtures of TBE and α_2-adrenergic agonists that have sedative, muscle-relaxant, and analgesic properties (Fish *et al.*, 2008). These particular strains were selected because they are among the outbred and inbred strains most widely used in biomedical research (Hedrich and Bullock, 2004).

The first aim of this study was to evaluate the anesthetic efficacy and safety of a low dose (200 mg/kg) of TBE in mixtures with α_2-adrenergic agonists. The second objective was to determine the different anesthetic effects of the TBE mixtures on the ICR, C57BL/6, and BALB/c strains, to help develop appropriate dose ratios for these various combinations.

Materials and Methods

Animals

Specific pathogen-free male Bkl:BKW-BKW (ICR) mice (body weight 20-29 g; 4-6 weeks old), C57BL/6/Bkl mice (body weight 18-23 g; 4-6 weeks old), and BALB/c/Bkl mice (body weight 19-22 g; 4-6 weeks old) were obtained from NaraBiotech (Pyeongtaek, Korea). All mice were verified free of contagious antibodies, 11 viruses, 15 bacteria, fungi, endoparasites, and ectoparasites prior to shipping by the vendor. After their arrival at our facility, we did not perform health monitoring of the mice. They were housed in a barrier

facility using an individually ventilated caging system (190W ×240L×170H mm) with beta-chip bedding on a 12:12 hr light-dark cycle (8 a.m. to 8 p.m.). The room temperature was kept at 23±1°C, with relative humidity of 50±5% and with 25 complete changes of filtered air per hr. Mice were divided into five mice per cage. Gamma-sterilized formula-m07 food (Feedlab Korea Co., Hanam, Korea) and autoclaved tap water were provided by bottle *ad libitum*. All experimental procedures were carried out according to a protocol approved by the Animal Care and Use Committee of Konkuk University.

TBE preparation

Commercially available 2,2,2-tribromoethanol (TBE) powder (T48402, Sigma-Aldrich, St. Louis, MO, USA) from the same lot number was used for all studies. To prepare a 1 g/mL stock solution, a measured amount of TBE powder was placed into a 50 mL polypropylene conical tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA) that was wrapped in aluminum foil. A micropipette was used to add *tert*-amyl-alcohol (240486, Sigma-Aldrich), and the solution was vortexed until somewhat dissolved at room temperature (25°C). To prepare fresh dilutions of TBE (200 and 400 mg/kg) in injection volumes of 0.35-0.50 mL, an appropriate volume of sterilized phosphate-buffered saline (P5368, Sigma-Aldrich) was added to the stock solution and vortexed until the entire stock solution dissolved in PBS at 25°C. Working solutions were sterilely filtered with a 0.20 µm disposable syringe filter unit (Mixed Cellulose Ester, ADVANTEC®, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Through the filtering procedure, working solutions were contained in sterilized glass tubes capped with rubber stoppers and wrapped in aluminum foil. Glass vials were autoclaved, and all glassware was triple-rinsed with distilled water before autoclave (the preparer wore gloves). We used freshly made TBE solution for all studies.

Anesthetic administration and monitoring

All anesthetic regimens were administered to study animals via a single intraperitoneal injection in the lower-right quadrant of the abdomen using a 26-gauge 1 mL syringe (Miner *et al.*, 1969; Coria-Avila *et al.*, 2007). Prior to administration, animals were weighed and doses of anesthetic agents were determined on a mg/kg basis. Each animal was placed back in its home cage until it lost its righting reflex, after which mice were laid on temperature-controlled heating platform (38°C; JeungDo B&P, Seoul, Korea). Eye ointment (Solco®, Hanlim Pharm. Co., Ltd., Seoul, Korea) was used to lubricate the outer surface of the eye. Induction time was considered to be from the time of the injection to the time of loss of the righting reflex and hindlimb pedal withdrawal reflex (toe-pinch response), indicating loss of consciousness (Arras *et al.*, 2001; Buitrago *et al.*, 2008). Anesthetic duration (surgical tolerance) was defined as the time between the loss and return of reflexes. Recovery time was the time from when the animal regained these reflexes to when the animal was able to right itself and ambulate. To apply pressure uniformly, the pedal withdrawal reflex was assessed using a Touch-Test™ Sensory Evaluator (North Coast Medical, Inc., San Jose, CA, USA) with a target force of 300 g, which indicates a deep pressure sensation, every 5 min (Lieggi *et al.*, 2005a). A clear attempt to withdraw the limb was judged a positive reaction. All animals breathed room air for the duration of the experiment.

Details of the anesthetic protocols are given in Table 2. Approximate initial dosages and ratios of the components were determined on the basis of previous experience and a small pilot study on ICR mice (data not shown). First, all the mice, including all the mouse strains, were assigned randomly to four groups, and then serially numbered. The animals were weighed and one of the anesthetic combinations (protocols 1 to 4; Table 2) was administered.

Evaluation of the efficacy of alternative combinations to C57BL/6 and BALB/c

To induce the proper anesthetic effect, alternative combinations to C57BL/6/Bkl and BALB/c/Bkl were determined on the basis of initial dose and a small pilot study (data not shown). C57BL/6/Bkl mice were assigned randomly to one group. BALB/c/Bkl mice were assigned two groups and serially numbered. Ten animals from each group were treated: C57BL/6/Bkl mice with protocol 5 and BALB/c/Bkl mice with protocols 6-7 (Table 2).

Pathologic evaluation

For protocols 1 through 7, 5 mice per test group were evaluated for pathology at 1 and 10 days post-injection (dpi). For sham control, 2 and 3 mice were evaluated at 1 and 10 dpi, respectively. Mice were monitored for clinical signs of illness. Mice were humanely euthanized via exsanguination under anesthesia. Isoflurane (Forane®, Choongwae Pharma Co., Seoul, Korea) was delivered by Vaporizer 100 series (Surgivet, Waukesha, WI, USA). A necropsy was performed and abnormalities noted. In cases with observed abnormalities, the stomach, small intestine, large intestine, liver, spleen, and abdomen wall were collected for histopathological evaluation. Tissues were fixed in 10% neutral buffered formalin. The specimens were embedded in paraffin wax and sections 4-5 µm in thickness were stained with hematoxylin and eosin.

Table 2. Summary of experiments and number of animals used

Protocol	Substance	Dose (mg/kg)	Number of animals		
			ICR	C57BL/6	BALB/c
sham	PBS	0.5 mL/animal	5	5	5
P1	1.25% TBE	200	10	10	10
P2	2.50% TBE	400	10	10	10
P3	1.25% TBE+X	200+10	10	10	10
P4	1.25% TBE+M	200+0.5	10	10	10
P5	1.25% TBE+X	200+20	ND	10	ND
P6	1.25% TBE+X	300+10	ND	ND	10
P7	1.25% TBE+M	200+1	ND	ND	10

PBS, phosphate-buffered saline; TBE, tribromoethanol; X, xylazine (Rompun®, Bayer HealthCare, Seoul, Korea); M, medetomidine (Domitor®, Pfizer Animal Health Korea Ltd., Seoul, Korea); ND, not done.

Statistical analysis

All statistical analyses were carried out using SPSS version 14.0K and GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). All data were presented as mean±SEM. One-way analysis of variance (ANOVA) was performed with Tukey's adjustment for multiple comparisons, to evaluate differences among the 3 mouse strains for induction time, anesthetic duration, and recovery time. A value $P<0.05$ was considered significant.

Results

Clinical signs

By day 10 following anesthetic administration, all strains in protocol 2 manifested the following abnormal signs: dehydration, disheveled appearance, porphyrin staining of the eyes and nose, and hunched posture. There were no abnormal clinical signs in other protocols, except protocol 2.

Evaluation of the efficacy of anesthesia protocols in different mouse strains

Protocol 1 in all strains produced ataxia and mild sedation. All animals in this protocol had clear signs of only light anesthesia with a strong pedal withdrawal reflex. Average durations of induction, anesthesia, and recovery are shown in Table 3. Four hundred mg/kg TBE in protocol 2 was a reliable anesthetic that provided surgical anesthesia for approximately 31-39 min in ICR and C57BL/6/Bkl mice, but for a relatively short time (08:48, min:sec) in BALB/c/Bkl mice. The combination of 200 mg/kg TBE and 10 mg/kg xylazine (protocol 3) induced the proper anesthetic depth in ICR and C57BL/6/Bkl mice, but not in BALB/c/Bkl mice. The average anesthetic time for BALB/c/Bkl mice (17:39) was significantly shorter than those for ICR (65:38) and C57BL/6/Bkl mice (73:59).

Evaluation of efficacy of alternative combinations to C57BL/6 and BALB/c

To increase the efficacy of anesthesia, alternative combinations (protocols 5 to 7) were examined (Table 3). In protocol 5, a higher dose of xylazine was tried on C57BL/6/Bkl mice. This combination was more successful than the original dose of protocol 3. In protocol 3, however, none of the BALB/c/Bkl mice reached surgical tolerance, even though the xylazine dose was increased by 30 mg/kg (data not shown). When an increased dose of TBE was tried on BALB/c/Bkl mice (protocol 6), 80% of animals reached the proper anesthetic depth, but the death rate was 20%. To increase the efficacy of protocol 4 with BALB/c/Bkl mice, the original medetomidine dose was increased 2-fold. This dose (protocol 7) provided a longer anesthetic duration, as well as higher anesthetic efficacy, than did the original dose of protocol 4 with a similar total sleeping time (anesthetic and recovery duration). In addition, 100% of animals reached surgical tolerance, and all animals survived protocol 7.

Pathologic evaluation

Grossly, 2 ICR mice in protocol 2 had evidence of ileus and thin abdominal walls at 1 dpi. Four ICR mice in protocol 2 had focally extensive areas of fibrous adhesions between the abdominal wall, cecum, and small intestine, which were filled with gas and/or fluid at 10 dpi (Figure 1B). Among the C57BL/6/Bkl mice in protocol 2, 5 mice at 1 dpi and four mice at 10 dpi had various degrees of distension in the gastrointestinal tract, supporting a gross description of ileus, and thin abdominal walls (Figure 1C). Among the BALB/c/Bkl mice in protocol 2, 4 mice at 1 dpi and five mice at 10 dpi showed abdominal lesions similar to other strains (Figure 1D). Upon microscopic examination, the ICR, C57BL/6/Bkl, and BALB/c/Bkl mice in protocol 2 had serosa of the stomach

Table 3. Efficacy and safety of anesthesia after administration of each different regimes

		P2[a]			P3[b]			P4[c]			P5[d]	P6[e]	P7[f]
		ICR	C57BL/6	BALB/c	ICR	C57BL/6	BALB/c	ICR	C57BL/6	BALB/c	C57BL/6	BALB/c	BALB/c
ANES rate (n/n)		10/10	10/10	4/10	10/10	10/10	0/10	8/10	10/10	7/10	10/10	8/10	10/10
Mean ±SEM (mm:ss)	IND[g]	01:21 ±00:07	01:39 ±00:05	03:51 ±00:26*†	02:53 ±00:32	03:18 ±00:36	NA	02:54 ±00:46	02:04 ±00:16	07:10 ±00:41*†	03:23 ±00:19	03:30 ±00:23	04:16 ±00:25
	ANES[h]	39:05 ±03:01	31:02 ±03:31	08:48 ±01:24*†	39:28 ±05:08	13:26 ±00:56*	NA	65:38 ±06:40	73:59 ±07:34*	17:39 ±04:06*†	27:03 ±04:28	59:15 ±06:37	24:43 ±02:53
	REC[i]	26:45 ±04:41	51:31 ±02:54*	27:29 ±04:47†	09:13 ±03:00	18:49 ±02:12*	NA[j]	48:28 ±08:51	42:26 ±05:21*	73:04 ±05:31†	23:34 ±02:38	41:31 ±06:23	57:35 ±05:58
Death rate		0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	2/10	0/10
Gross lesion	1 dpi[k]	2/5	5/5	5/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4	0/5
	10 dpi	4/5	4/5	4/5	0/5	0/5	0/5	0/4	0/5	0/5	0/5	0/4	0/5

Data are presented as mean values and standard errors of anesthetized animals. [a]tribromoethanol (TBE) 400 mg/kg; [b]TBE 200 mg/kg+xylazine 10 mg/kg; [c]TBE 200 mg/kg+medetomidine 0.5 mg/kg; [d]TBE 200 mg/kg+xylazine 20 mg/kg; [e]TBE 300 mg/kg+xylazine 10 mg/kg; [f]TBE 200 mg/kg+medetomidine 1 mg/kg; [g]induction time; [h]anesthetic duration; [i]recovery time; [j]not applicable (strong pedal withdrawal reflex); [k]days of post injection; **P*<0.05 versus values for ICR group at the same protocol; †*P*<0.05 versus values for C57BL/6 at the same protocol (one-way ANOVA, Tukey-Kramer multiple comparisons test).

and small intestine, which had mononuclear cells intermixed with the fibrous tissue (Figure 2). There was no inter-strain variability in the gross or histopathological changes that would be considered as TBE side effects.

Discussion

The selection of an anesthetic regimen for use in research depends on the strains of animal as well as species (Arras *et al.*, 2001). Zeller *et al.* (1998) reported that no strain-specific difference was observed in strains ICR, OF-1, or NMRI, whereas our results showed that there were significant differences in anesthetic and recovery time, depending on the strain of mice (ICR, C57BL/6/Bkl, and BALB/c/Bkl in this study).

The dose of TBE used in protocol 2 was based on doses reported in the literature (Gardner *et al.*, 1995; Zeller *et al.*, 1998; Thompson *et al.*, 2002). While the lowest dose of TBE that resulted in adequate anesthesia, based on the toe-pinch reflex, was a dose of 375 mg/kg, the lowest dose that consistently produced adequate anesthesia in all ICR mice tested was 400 mg/kg (Lieggi *et al.*, 2005a). However, fibrous adhesions between the abdominal wall, cecum, and small intestine, which were filled with gas and/or fluid, were examined in all mice strains for protocol 2.

TBE is unavailable as a pharmaceutical-grade compound, and its use is approved only under stringent guidelines (Fish *et al.*, 2008). Some researchers concluded that the reported side effects were most likely due to improper mixing and storage of the compound, as well as, inadequate anesthetic support (Lieggi *et al.*, 2005a, 2005b). Although the ICR, C57BL/6/Bkl, and BALB/c/Bkl mice in protocol 2, using high-dose TBE, had evidence of serosa with severe fibrosis intermixed with mononuclear cells, those in protocols using the low-dose TBE did not showed any histopathological change or serositis. It is known that these side effects are induced by TBE, per se, but are not related to the solvent used (Zeller *et al.*, 1998; Reid *et al.*, 1999). Results from our study confirm that the extent of the necrotic and inflammatory changes show TBE's concentration-dependent effects. In conclusion, for long-term studies, a therapeutic concentration (400 mg/kg) of TBE cannot be recommended for intraperitoneal use in mice without regard to its anesthetic efficacy.

Peritoneal insults such as anesthetic injection are external stimuli. They induce an inflammatory cascade, subsequently causing a reduction in gastrointestinal motility (Person and Wexner, 2006). In the present study, therefore, local inflammation in the peritoneal cavity could be postulated as the cause for reduced motility. In addition, fibrinolytic enzymes readily lyse fibrin in the normal peritoneal cavity, but inflammation inactivates this system (Hall *et al.*, 1998). Persistence of intra-abdominal fibrin subsequently will cover the gut with fibrinopurulent exudates that may develop into a fibrinous peel and eventually adhesions (van Goor *et al.*, 1994). In the present study, therefore, a fibrous adhesion of abdominal organs in mice that received 400 mg/kg TBE was considered the outcome of peritonitis.

The α_2-adrenergic agonists (xylazine and medetomidine) are potent sedatives with variable muscle relaxant and analgesic activity (Green, 1975). Although TBE has been used effectively

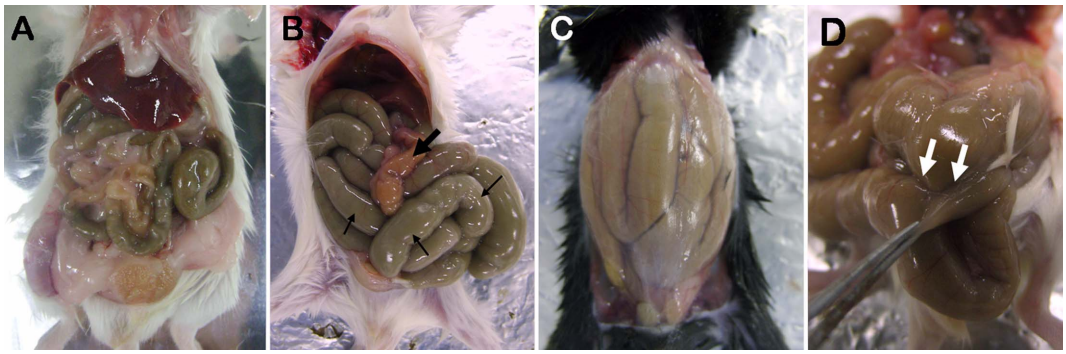

Figure 1. Appearance of a mouse's abdominal cavity at 10 dpi. (A) Gross necropsy of ICR mice that received saline intraperitoneally. Normal appearance of the abdominal cavity is noted. (B) Gross necropsy of ICR mouse that received 400 mg/kg tribromoethanol (TBE) intraperitoneally. The stomach is grossly empty (thick, dark arrow). The intestine is distended with fluid and digested food, supporting a gross description of ileus (thin, dark arrow). (C) Thin abdominal wall and distension of the gastrointestinal tract found upon gross necropsy of C57BL/6 mouse that was administered 400 mg/kg TBE. (D) BALB/c mouse that received 400 mg/kg TBE showed focally extensive area of fibrous adhesion between the small intestine and cecum, which were filled with digested food (open arrow).

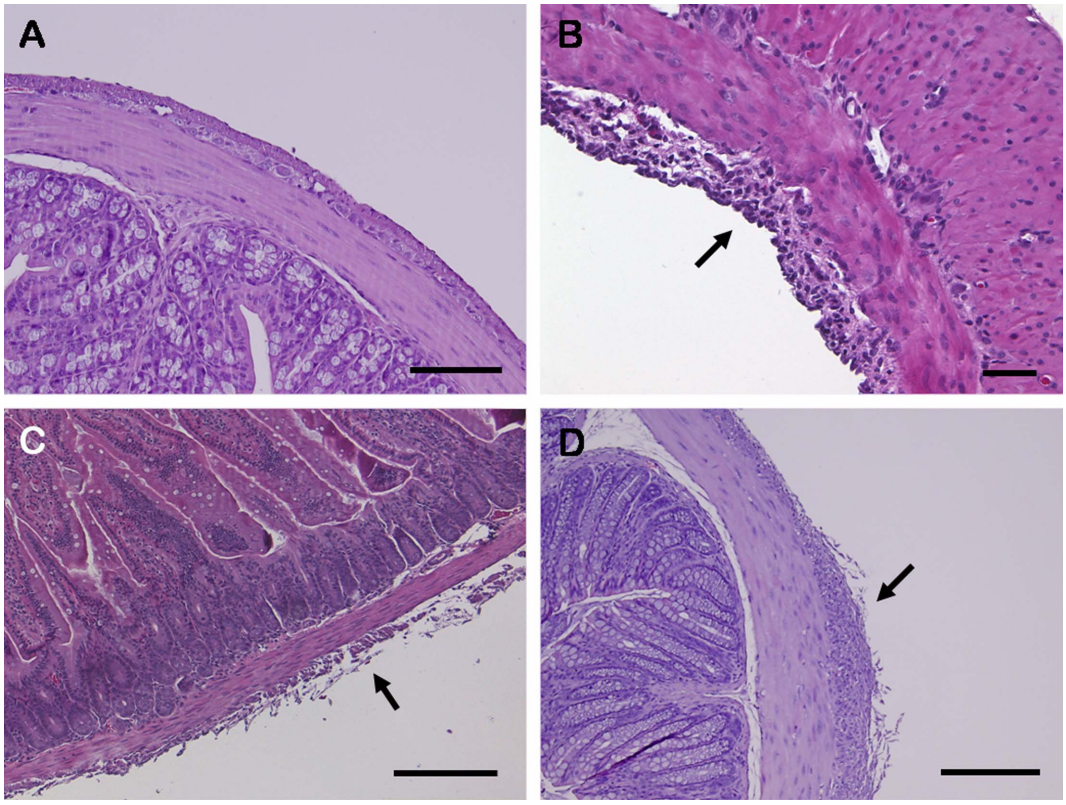

Figure 2. Microscopic photographs of gastrointestinal serosa in mice at 10 dpi. (A) Normal histology of large intestine from ICR mouse that was injected with saline intraperitoneally (bar, 100 μm). (B) Histopathology of a stomach from ICR mouse that received 400 mg/kg tribromoethanol (TBE) intraperitoneally. Section demonstrates intermixed mononuclear cells with the fibrous tissue in the serosa (arrow). Hematoxylin and eosin stain; bar, 50 μm (C) Histopathology of the large intestine from a C57BL/6 mouse that received 400 mg/kg TBE intraperitoneally. The section demonstrates fibrosis and inflammation of the serosa (arrow). Hematoxylin and eosin stain; bar, 200 μm. (D) Histopathology of the large intestine from a BALB/c mouse that was administered 400 mg/kg TBE intraperitoneally. This large intestine section demonstrates mononuclear cells intermixed with the fibrous tissue (arrow). Hematoxylin and eosin stain; bar, 200 μm.

for years in many labs, mixtures of TBE and α_2-adrenergic agonists are suitable alternatives to using TBE alone, and have fewer side effects. Such mixtures produced adequate anesthesia in the protocols studied here, although mixtures of TBE and α_2-adrenergic agonists are associated with less inflammation in gross and histopathological lesions when

compared to the use of TBE alone. Another advantage of combinations of low-dose TBE and α_2-adrenergic agonists over high-dose TBE is that both xylazine and medetomidine are known to induce analgesia. The analgesic effects and mechanisms of action for TBE are not exactly known (Lieggi *et al.*, 2005a), and TBE-involved $GABA_A$-agonist injectable anesthetics are speculated to be poor analgesics (Fish *et al.*, 2008).

In summary, these findings suggest that the single-use of 400 mg/kg TBE is not a suitable anesthetic for major surgery in all mice strains, because of the resulting pathologic changes in the abdominal organs. It was found that the combination of low-dose TBE and α_2-adrenergic agonists appeared to induce safe and satisfactory anesthesia. Finally, obvious variations in anesthetic efficacy were found among the ICR, C57BL/6/Bkl, and BALB/c/Bkl mouse strains.

In future, to further understand the underlying pathophysiological changes, we will examine the level of cytokines in protein levels, through ELISA experiments with abdominal lavage fluid and spleen fluid collected from mice treated with low-dose TBE.

Acknowledgments

This work was supported by the Korea Research Foundation Grant and funded by the Korean Government (MOEHRD, Basic Research Promotion Fund, KRF-2007-331-E00261).

References

Arras, M., Autenried, P., Rettich, A., Spaeni, D. and Rulicke, T. (2001) Optimization of intraperitoneal injection anesthesia in mice: Drugs, dosages, adverse effects, and anesthesia depth. *Comp. Med*. 51(5), 443-456.

Bette, M., Schlimme, S., Mutters, R., Menendez, S., Hoffmann, S. and Schulz, S. (2004) Influence of different anaesthetics on pro-inflammatory cytokine expression in rat spleen. *Lab. Anim.* 38(3), 272-279.

Buitrago, S., Martin, T.E., Tetens-Woodring, J., Belicha-Villanueva, A. and Wilding, G.E. (2008) Safety and efficacy of various combinations of injectable anesthetics in BALB/c mice. *J. Am. Assoc. Lab. Anim. Sci*. 47(1), 11-17.

Coria-Avila, G.A., Gavrila, A.M., Menard, S., Ismail, N. and Pfaus, J.G. (2007) Cecum location in rats and the implications for intraperitoneal injections. *Lab. Anim. (NY)* 36(7), 25-30.

Fish, R.E. and Meyer, R.E. (2008) Pharmacology of injectable anesthetics, sedatives, and tranquilizers. In *Anesthesia and Analgesia in Laboratory Animals* (Fish, R.E. ed.), 2nd ed., pp. 27-82, Academic Press, Burlington.

Flecknell, P.A. (2005) *Laboratory Animal Anaesthesia*, pp. 160-171, Elsevier Academic Press, San Diego.

Gardner, D.J., Davis, J.A., Weina, P.J. and Theune, B. (1995) Comparison of tribromoethanol, keta-mine/acetylpromazine, telazol/xylazine, pentobarbital, and methoxyflurane anesthesia in HSD:ICR mice. *Lab. Anim. Sci.* 45(2), 199-204.

Gopalan, C., Hegade, G.M., Bay, T.N., Brown, S.R. and Talcott, M.R. (2005) Tribromoethanol-medetomidine combination provides a safe and reversible anesthetic effect in Sprague-Dawley rats. *Contemp. Top Lab. Anim. Sci*. 44(1), 7-10.

Green, C.J. (1975) Neuroleptanalgesic drug combinations in the anaesthetic management of small laboratory animals. *Lab. Anim*. 9(3), 161-178.

Hall, J.C., Heel, K.A., Papadimitriou, J.M. and Platell, C. (1998) The pathobiology of peritonitis. *Gastroenterology* 114(1), 185-196.

Hedenqvist, P. and Hellebrekers, L.J. and Van Hoosier, G.L. (2003) Laboratory animal analgesia, anesthesia, and euthanasia. In *Handbook of Laboratory Animal Science* (Hau, J. ed.), vol. I, 2nd ed., pp. 413-457, CRC Press, Boca Raton.

Hedrich, H.J. and Bullock, G.R. (2004) *The Laboratory Mouse*, pp. 543-554, Elsevier Academic Press, San Diego.

Kiatchoosakun, S., Kirkpatrick, D. and Hoit, B.D. (2001) Effects of tribromoethanol anesthesia on echocardiographic assessment of left ventricular function in mice. *Comp. Med*. 51(1), 26-29.

Lieggi, C.C., Artwohl, J.E., Leszczynski, J.K., Rodriguez, N.A., Fickbohm, B.L. and Fortman, J.D. (2005a) Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice. *Contemp. Top Lab. Anim. Sci*. 44(1), 17-22.

Lieggi, C.C., Fortman, J.D., Kleps, R.A., Sethi, V., Anderson, J.A., Brown, C.E. and Artwohl, J.E. (2005b) An evaluation of preparation methods and storage conditions of tribromoethanol. *Contemp. Top Lab. Anim. Sci*. 44(1), 11-16.

Mascia, M.P., Machu, T.K. and Harris, R.A. (1996) Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. *Br. J. Pharmacol.* 119(7), 1331-1336.

Miner, N.A., Koehler, J. and Greenaway, L. (1969) Intraperitoneal injection of mice. *Appl. Microbiol.* 17(2), 250-251.

National Research Council (1996) *Guide for the Care and Use of Laboratory Animals*, pp. 56-70, National Academy Press, Washington DC.

Person, B. and Wexner, S.D. (2006) The management of postoperative ileus. *Curr. Probl. Surg*. 43(1), 6-65.

Reid, W.C., Carmichael, K.P., Srinivas, S. and Bryant, J.L. (1999) Pathologic changes associated with use of tribromoethanol (avertin) in the Sprague Dawley rat. *Lab. Anim. Sci*. 49(6), 665-667.

Tarin, D. and Sturdee, A. (1972) Surgical anaesthesia of mice: Evaluation of tribromoethanol, ether, halothane and methoxyflurane and development of a reliable technique. *Lab. Anim*. 6(1), 79-84.

Thompson, J.S., Brown, S.A., Khurdayan, V., Zeynalzadedan, A., Sullivan, P.G. and Scheff, S.W. (2002) Early effects of tribromoethanol, ketamine/xylazine, pentobarbitol, and isoflurane anesthesia on hepatic and lymphoid tissue in ICR mice. *Comp. Med*. 52(1), 63-67.

Ticku, M.K. and Kulkarni, S.K. (1988) Molecular interactions of ethanol with GABAergic system and potential of RO15-4513 as an ethanol antagonist. *Pharmacol. Biochem. Behav.* 30(2), 501-510.

van Goor, H., de Graaf, J.S., Grond, J., Sluiter, W.J., van der Meer, J., Bom, V.J. and Bleichrodt, R.P. (1994) Fibrinolytic activity in the abdominal cavity of rats with faecal peritonitis. *Br. J. Surg.* 81(7), 1046-1049.

Weiss, J. and Zimmermann, F. (1999) Tribromoethanol (avertin) as an anaesthetic in mice. *Lab. Anim*. 33(2), 192-193.

Zeller, W., Meier, G., Burki, K. and Panoussis, B. (1998) Adverse effects of tribromoethanol as used in the production of transgenic mice. *Lab. Anim*. 32(4), 407-413.