Effects of pentobarbital, isoflurane, or medetomidine–midazolam–butorphanol anesthesia on bronchoalveolar lavage fluid and blood chemistry in rats

Yasuhiro Tsubokura1, Toshio Kobayashi1, Yutaka Oshima1, Naoki Hashizume2, Makoto Nakai3, Shozo Ajimi1 and Nobuya Imatanaka2

1CERI Hita, Chemicals Evaluation and Research Institute, Japan, 3-822, Ishii-machi, Hita-shi, Oita 877-0061, Japan
2CERI Kurume, Chemicals Evaluation and Research Institute, Japan, 3-2-7, Miyanojin, Kurume-shi, Fukuoka 839-0801, Japan
3Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute, Japan, 1-4-25, Kouraku, Bunkyo-ku, Tokyo 112-0004, Japan

(Received May 23, 2016; Accepted July 12, 2016)

ABSTRACT — Bronchoalveolar lavage fluid (BALF) is commonly examined for pulmonary toxicity in animal studies. Two common means of anesthesia before euthanasia and bronchoalveolar lavage in rats are intraperitoneal injection of pentobarbital and inhalation of isoflurane. Medetomidine–midazolam–butorphanol is an alternative anesthesia to pentobarbital for animal welfare; however, the effect of this combination on BALF and blood chemistry is unknown. Here, we compared the effects of anesthesia by intraperitoneal injection of pentobarbital or one of two combinations of medetomidine–midazolam–butorphanol (dose, 0.375-2.0-2.5 or 0.15-2.0-2.5 mg/kg) or by inhalation of isoflurane on BALF and blood chemistry in rats with or without pulmonary inflammation. In BALF, we determined total protein, albumin, lactate dehydrogenase, total cell count and neutrophil count. In serum, we conducted a general chemistry screen. After anesthesia with pentobarbital or isoflurane, there were no significant differences between any of the BALF or blood chemistry parameters with or without inflammation. After anesthesia with either of the combinations of medetomidine–midazolam–butorphanol, lactate dehydrogenase, total cell count, neutrophil count, and almost all of the blood chemistry parameters were comparable with those observed after pentobarbital or isoflurane; however, BALF albumin and serum glucose were significantly increased in rats without inflammation. After the combination of low-dose medetomidine in rats with inflammation, BALF parameters were comparable with those observed after pentobarbital or isoflurane. Our results show that, of the anesthetics examined, inhalation of isoflurane is the most appropriate means of anesthesia when examining BALF or serum for toxicity studies in rats.

Key words: BALF, Blood chemistry, Isoflurane, Medetomidine, Pentobarbital

INTRODUCTION

Intraperitoneal (i.p.) injection of pentobarbital (Roberts et al., 2013; Sager et al., 2008; Sung et al., 2011; Wako et al., 2010) and inhalation of isoflurane (Takehara et al., 2014; Haberl et al., 2013; Lim and Chung, 2014; Lim et al., 2012, 2013) are common means of anesthetizing rats before euthanasia and bronchoalveolar lavage (BAL). Isoflurane has been shown to be very safe in rats due to a great margin of safety (Cesarovic et al., 2010; Gaertner et al., 2008), whereas pentobarbital has a narrow margin of safety and provides minimal analgesic effect (Meyer and Fish, 2008; Olfert et al., 1993). Therefore, as an alternative to pentobarbital in studies in rat, a combination of medetomidine–midazolam–butorphanol has been suggested (Kirihera et al., 2016). The multidrug combination of medetomidine–midazolam–butorphanol does not include narcotics ingredients like ketamine and has both sedative and analgesic actions. Medetomidine is an α2-adrenergic receptor agonist (Meyer and Fish, 2008; Sinclair, 2003). Midazolam is a water-soluble benzodiazepine that produces marked sedation in rodents.
Butorphanol is a synthetic opioid agonist–antagonist with analgesic action (Heavner and Cooper, 2008). It was reported that the induction time for anesthesia was short, approximately 10 min, and anesthetic duration was at least 25 min by the multi-drug combination of medetomidine–midazolam–butorphanol (Kirihara et al., 2016; Senoh et al., 2016). Senoh et al. examined that the multi-drug combination of medetomidine–midazolam–butorphanol has been examined to be applied to euthanasia before BAL fluid (BALF) sampling (Senoh et al., 2016), but the examined combination ratio of the anesthesia was limited and the effect of the anesthesia on BALF has not been investigated enough.

Examination of the cellular and chemical contents of BALF is a common means of evaluating the pulmonary toxicity of chemicals or drugs in inhalation (Lim and Chung, 2014; Lim et al., 2012, 2013; Sung et al., 2011) or intratracheal administration studies in rats (Haberl et al., 2013; Roberts et al., 2013; Saget et al., 2008; Wako et al., 2010). However, because most anesthetics induce metabolic changes in rats, it is likely that the cellular and chemical contents of BALF, as well as blood chemistry, are affected by the anesthetic administered before euthanasia and BAL. Furthermore, in inflamed lungs, because inhaled anesthetics (unlike injected anesthetics) come into direct contact with the respiratory system, it is likely that inhaled anesthetics make inflammatory parameters of BALF worse compared to injected anesthetics. Therefore, it is important to elucidate the changes induced by different anesthetics so that we can effectively compare the values of BALF and blood chemistry parameters obtained with different methods of anesthesia.

Here, we examined the effects of different anesthetics on BALF and blood chemistry parameters in rats. First, we examined the effects in untreated rats by administering one of two anesthetics or one of two doses of anesthetic combinations (i.e., i.p. injected pentobarbital, i.p. injected medetomidine–midazolam–butorphanol [low- or high-dose medetomidine], or inhaled isoflurane) before euthanasia and then comparing the BALF and blood chemistry parameters among anesthetic groups. Then, in rats in which pulmonary inflammation had been induced by intratracheal administration of nickel oxide (NiO) and in vehicle controls that had received intratracheal instillation of purified water, we examined the effects of administration of one of three anesthetics or anesthetic combinations (i.e., i.p. injected pentobarbital, i.p. injected medetomidine–midazolam–butorphanol [low-dose medetomidine] or inhaled isoflurane) before euthanasia and compared the BALF parameters.

**MATERIALS AND METHODS**

**Animals**

Male F344/DuCrIcrlJ rats were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). At the time of anesthesia, the animals were 13 weeks old and weighed from 224.3 to 266.5 g. The animals were housed in barrier-system animal rooms maintained at 21°C to 25°C with relative humidity 40% to 70%, 10 to 15 air changes per hour, and a photoperiod of 12 hr of light per day (lights on at 7:00 and off at 19:00). The study was complied with the our laboratory’s guideline for the animal experiment which referred to the Law Concerning the Protection and Control of Animals (1973) and guidelines such as Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Ministry of Education, Japan, 2006) and approved by our laboratory’s Institutional Animal Care and Use Committee before the start of the experiment.

**Anesthetic agents and regimens**

The anesthetic agents and regimens are described below and the dose, concentration and induction time are listed in Table 1. Pentobarbital (Somnopentyl; Kyoritsu

<table>
<thead>
<tr>
<th>Table 1. Dose, concentration and induction time of each anesthesia method in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anesthesia method</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Pentobarbital</td>
</tr>
<tr>
<td>Isoflurane</td>
</tr>
<tr>
<td>MMB1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MMB2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Vol. 41 No. 5
Effects of anesthetics on BALF parameters in rats with pulmonary inflammation

Ten rats were used for each anesthetic (5 as treatment to induce pulmonary inflammation and 5 as vehicle control). To induce pulmonary inflammation, 2 mg/mL NiO (US3352, US Research Nanomaterials, Inc., Houston, TX, USA) was suspended in purified water (Takasugi Pharmaceutical Co., Ltd., Fukuoka, Japan) at a volume of 1.0 mL/kg BW was intratracheally instilled via a spraysonde (MicroSprayer Aerosolizer, Penn-Century, Inc., Philadelphia, PA, USA) under isoflurane anesthesia. Isoflurane (Escaim; Mylan Seiyaku Ltd., Tokyo, Japan) was administered by inhalation at a concentration of 3.5% with oxygen as a career gas for 4 min in a sealed chamber at a pressure of 6 psi by using inhalation anesthesia apparatus (RC2 rodent circuit controller, VetEquip, Inc., Livermore, Canada) at room temperature to induce anesthesia.

Multiple-drug combination 1 (MMB1): medetomidine hydrochloride (Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), midazolam (Sandoz K.K., Tokyo, Japan), and butorphanol (Meiji Seika Pharma Co., Ltd., Tokyo, Japan) were combined and diluted by physiological saline for injection. Then the mixture was administered i.p. at a dose of medetomidine hydrochloride (0.15 mg/kg BW), midazolam (2 mg/kg BW) and butorphanol (2.5 mg/kg BW) (Kirihara et al., 2016). The animals were kept at room temperature for 15 to 25 min after the administration to induce anesthesia. Multiple-drug combination 2 (MMB2): medetomidine hydrochloride, midazolam, and butorphanol were combined and diluted by physiological saline for injection. Then the mixture was administered i.p. at a dose of medetomidine hydrochloride (0.375 mg/kg BW), midazolam (2 mg/kg BW) and butorphanol (2.5 mg/kg BW) (Senoh et al., 2016). The animals were kept at room temperature for 15 to 25 min after the administration to induce anesthesia.

Effects of anesthetics on BALF and blood chemistry in rats without pulmonary inflammation

Five rats were used for each anesthetic. The animals were fasted for 16 to 19 hr and then anesthetized with pentobarbital, isoflurane, MMB1, or MMB2. After the anesthesia was induced, a blood sample had been drawn from the abdominal aorta, the animal was euthanized by bleeding from the abdominal aorta. Approximately 10 min after the bleeding, BALF was collected as described below. Serum for blood chemistry was obtained by centrifugation of the blood sample. The values of BALF and blood chemistry parameters were determined as described below.

BAL
After euthanasia by bleeding was complete, the trachea of each rat was exposed and a cannula was inserted via ventral incision and ligation of the trachea. Saline (7 mL; Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) was instilled into the whole lung at a pressure of 2900 Pa (30 cmH₂O) and drained by gravity. This lavage procedure was performed twice (i.e., a total of 14 mL of saline was instilled; > 90% was recovered) and the recov-
ered fluids were combined. The number of total cells in BALF (total cell count) was determined by using an ADVIA 120 hematology analyzer (Siemens Healthcare Diagnostics Inc., New York, NY, USA). The concentrations of total protein, and albumin, and lactate dehydrogenase activity (chemicals that reflect the inflammatory state) were determined in the BALF supernatant, which was obtained by centrifugation (400 × g, 10 min, 4°C), by using a Hitachi Automatic Clinical Analyzer 7170 (Hitachi Science Systems Ltd., Ibaraki, Japan). Cell pellets were resuspended in 1 mL of phosphate-buffered saline and a smear slide was prepared. After Pappenheim staining, the leukocyte differential was determined by counting 200 cells per rat. Finally, the number of neutrophils was calculated from the leukocyte differential and total cell count.

**Blood chemistry panel**

The concentrations of the following chemicals in serum were determined with a Hitachi Automatic Clinical Analyzer 7170 (Hitachi Science Systems Ltd.): aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, total cholesterol, triglyceride, blood urea nitrogen, creatinine, total protein, albumin, albumin-to-globulin ratio, glucose, total bilirubin, inorganic phosphorus, calcium, and total bile acid.

**Statistical analysis**

Statistical analyses were conducted to examine the differences between the BALF and serum parameters. First, Bartlett’s test for homogeneity of variance was used to determine differences among the groups. When no significant difference was found ($p > 0.05$) using Bartlett’s test, the parametric Tukey–Kramer test was conducted. When a significant difference was found ($p < 0.05$) using Bartlett’s test, the non-parametric Tukey–Kramer test was conducted. In the second experiment, the $F$ (variance ratio)-test was used to determine the difference between the vehicle control group and the NiO group. When there was no significant difference ($p > 0.05$), Student’s $t$-test was conducted. When there was a significant difference ($p < 0.05$), the Aspin–Welch $t$-test was conducted. Statistical significance was judged at the 0.05 probability level. StatLight software (Yukms Co., Ltd., Tokyo, Japan) was used for all the statistical analyses.

**RESULTS**

**Effects of anesthetics on BALF and blood chemistry in rats without pulmonary inflammation**

First we examined the effects of the anesthetics on BALF in untreated rats (Figs. 1A-1E). Mean albumin concentration in the rats administered MMB2 was significantly higher than that in the rats administered pentobarbital, isoflurane, or MMB1 ($p < 0.01$ compared with pentobarbital and isoflurane; $p < 0.05$ compared with MMB1, Fig. 1A). Mean albumin concentration was also higher in the rats administered MMB1 than in the rats administered pentobarbital or isoflurane; however, this difference was not statistically significant. Mean albumin concentration was comparable in rats given pentobarbital and those given isoflurane. Mean total protein concentration was significantly higher in rats administered MMB2 than in those administered isoflurane ($p < 0.05$, Fig. 1B). Total protein concentration was comparable in rats given pentobarbital, isoflurane and MMB1. Mean total cell count in the rats administered MMB2 was significantly lower than that in rats given MMB1 (Fig. 1C); however, this was considered an incidental change because the values remained within the range of our laboratory’s historical data (45.9 to 183.1 cells/μL: mean ± 2 × standard deviation obtained from 40 rats anesthetized with pentobarbital). For mean lactate dehydrogenase activity and neutrophil count, there were no significant differences between any of the groups (Figs. 1D, 1E).

Next, we examined the effects of the anesthetics on blood chemistry and found statistically significant differences for seven of the parameters examined (Figs. 2A-2G; data shown for these seven parameters only). Mean serum glucose concentration was significantly higher ($p < 0.01$) in the rats administered MMB2 than in the rats given pentobarbital, isoflurane, or MMB1 (Fig. 2A). Although mean serum glucose concentration in the rats administered MMB1 was lower than that in the rats administered MMB2, it was significantly higher than that in the rats given pentobarbital or isoflurane ($p < 0.01$ compared with pentobarbital; $p < 0.05$ compared with isoflurane). Mean serum alanine aminotransferase ($p < 0.05$), total protein ($p < 0.01$), alkaline phosphatase ($p < 0.05$), albumin ($p < 0.01$), and aspartate aminotransferase ($p < 0.05$) concentrations were all significantly higher in rats given MMB2 than in rats given pentobarbital (Figs. 2B-2F). Mean serum inorganic phosphorus level was significantly higher in rats administered MMB2 than in those given pentobarbital or isoflurane (Fig. 2G). However, all of the mean blood chemistry values of the animals administered
MMB2—with the exception of mean serum glucose concentration—were within the range of our historical data (alanine aminotransferase: 16 to 35 IU/L; total protein: 34 to 66 μg/mL; alkaline phosphatase: 261 to 540 IU/L; albumin: 16 to 32 μg/mL; aspartate aminotransferase: 38 to 80 IU/L; inorganic phosphorus: 5.5 to 9.3 mg/dL, mean ± 2 × standard deviation obtained from 40 rats anesthetized with pentobarbital); therefore, these changes were considered incidental. No statistically significant differences were detected between rats administered pentobarbital.

Fig. 1. Bronchoalveolar lavage fluid parameter values in untreated control rats. Differences among the groups were determined with the Tukey–Kramer test (*p < 0.05, **p < 0.01). TP, total protein; TCC, total cell count; LDH, lactate dehydrogenase; PB, pentobarbital; IF, isoflurane; MMB1, 0.15 mg/kg body weight (BW) medetomidine + 2 mg/kg BW midazolam + 2.5 mg/kg BW butorphanol; MMB2, 0.375 mg/kg BW medetomidine + 2 mg/kg BW midazolam + 2.5 mg/kg BW butorphanol.
Fig. 2. Blood chemistry values in untreated control rats. Differences among the groups were determined with the Tukey–Kramer test (* p < 0.05, ** p < 0.01). ALT, alanine aminotransferase; TP, total protein; ALP, alkaline phosphatase; AST, aspartate aminotransferase; IP, inorganic phosphorus; PB, pentobarbital; IF, isoflurane; MMB1, 0.15 mg/kg body weight (BW) medetomidine + 2 mg/kg BW midazolam + 2.5 mg/kg BW butorphanol; MMB2, 0.375 mg/kg BW medetomidine + 2 mg/kg BW midazolam + 2.5 mg/kg BW butorphanol.
bital or isoflurane for any of the blood chemistry parameters examined.

Effects of anesthetics on BALF in rats with pulmonary inflammation

To examine the effects of the anesthetics on rats with pulmonary inflammation, rats were instilled intratracheally with either NiO or purified water as vehicle control and the effects of the anesthetics on BALF were then examined (Figs. 3A-3E). Among vehicle control rats, mean albumin concentration was significantly higher in those given MMB1 than in those given pentobarbital or isoflurane (Fig. 3A); this was consistent with the results of the first experiment. In vehicle control rats, no significant differences were detected among the different anesthetics in terms of mean total protein, total cell count, lactate dehydrogenase, or neutrophil count (Figs. 3B-3E). In rats instilled with NiO, irrespective of the anesthetic administered, mean albumin, total protein, total cell count, lactate dehydrogenase and mean neutrophil count were all significantly greater ($p < 0.01$) than in rats of vehicle control (Figs. 3A-3E). However, no significant differences were detected among the different anesthetics in terms of any of the parameters examined.

DISCUSSION

We examined the effects of different anesthetics (i.e., i.p. injection of pentobarbital, i.p. injection of medetomidine–midazolam–butorphanol, or inhalation of isoflurane) on BALF parameters and blood chemistry in rats that had, or had not, undergone pulmonary instillation to induce pulmonary inflammation. We conducted both i.p. anesthesia of pentobarbital and medetomidine–midazolam–butorphanol by similar induction time, between 15 and 25 min, to compare the effects on blood and BALF with little difference of the duration from the anesthetic onset to blood or BALF sampling between these i.p. anesthesia. As the result, we detected no statistically significant differences in BALF or blood chemistry parameters after i.p. anesthesia with pentobarbital or isoflurane inhalation, regardless of whether or not pulmonary inflammation had been induced. After anesthesia with the combination of MMB1 and MMB2, there were no significant differences in BALF lactate dehydrogenase activity, total cell count, and neutrophil count, or in most of the blood chemistry values, compared with these values after pentobarbital or isoflurane anesthesia; however, BALF total protein concentration and albumin concentration and serum glucose concentration increased in rats without inflammation. After anesthesia with MMB1 in rats with inflammation, BALF parameters were comparable to those observed after pentobarbital or isoflurane anesthesia.

Because pentobarbital is a type A γ-aminobutyric acid (GABA_A) receptor agonist (Meyer and Fish, 2008; Steinbach and Akk, 2001), and isoflurane is reported to increase both the potency and efficacy of partial GABA_A receptor agonists (Topf et al., 2003), it is not surprising that the results for these two anesthetics were comparable in rats without inflammation. Besides, there were no significant differences in BALF parameters in rats with NiO-induced pulmonary inflammation that received these two anesthetics, despite the anesthesia being delivered via different routes. The exposure time and concentration (4 min; 3.5%) that we used here appears to have not been enough to induce changes in BALF. Our results suggest that the BALF parameter and blood chemistry data obtained after pentobarbital or isoflurane anesthesia in rats can be compared without concern for the different effects the anesthetics have on BALF or blood chemistry, given that all other test conditions are the same.

Mean serum glucose concentration and mean BALF total protein and albumin concentrations were higher in untreated control rats given MMB2 than in those given MMB1. Because MMB2 and MMB1 included the same concentrations of midazolam and butorphanol but MMB2 included 2.5 times the concentration of medetomidine (0.375 mg/kg BW) compared with MMB1 (0.15 mg/kg BW), it is likely that the higher mean serum glucose concentration and mean BALF total protein and albumin concentrations were due to the increased concentration of medetomidine, which is an α2-adrenergic receptor agonist (Sinclair, 2003). Indeed, increases in serum glucose concentration in rats have been reported with other multiple-drug combinations that have included medetomidine (Arnold and Langhans, 2010; Callahan et al., 2014). It has also been reported that medetomidine increases blood glucose concentrations in dogs and mice with significantly decreasing plasma insulin concentration (Guedes and Rude, 2013; Guedes et al., 2013). It is well known that α2-adrenergic receptor agonists inhibit the release of insulin via α2-adrenoceptors on pancreatic β-cells (Hillaire- Buys et al., 1985; Yamazaki et al., 1982). Therefore, the increase in serum glucose we observed here may be the result of a medetomidine-induced decrease in serum insulin concentration. Because we did not measure insulin concentrations, further studies are needed to elucidate the reason for the increase in serum glucose.

Generally, increases in BALF albumin and total protein concentration represent an increase in vascular permeability due to inflammation in the lung. However, the increases in albumin and total protein concentration we found
here after administration of MMB1 or MMB2 can be considered unrelated to inflammation, because these increases were observed in both untreated control rats and controls given purified water intratracheally. Medetomidine induces a transient increase in blood pressure owing to its effect on peripheral α2-adrenergic receptors (Ruskoaho and Leppäluoto, 1989; Sinclair, 2003). Therefore, the increases in BALF albumin and total protein concentrations may be due to leakage of albumin and other proteins from the blood into the alveoli during the medetomidine-mediated transient increase in blood pressure. Further studies directly examining the effects of combinations of medetomidine–midazolam–butorphanol on vasopermeability will be needed to elucidate the real reason for the increases in BALF albumin and protein concentrations observed here.

Fig. 3. Bronchoalveolar lavage fluid parameter values in rats instilled with purified water (VC) or nickel oxide (NiO) under isoflurane anesthesia. Bronchoalveolar lavage fluid was collected and examined 3 days after the intratracheal instillation. Differences between VC group and corresponding NiO group were determined with the Student’s t-test or the Aspin–Welch t-test (## p < 0.01). Differences among anesthesia groups were determined with the Tukey–Kramer test (* p < 0.05). TP, total protein; TCC, total cell count; LDH, lactate dehydrogenase; PB, pentobarbital; IF, isoflurane; MMB1, 0.15 mg/kg body weight (BW) medetomidine + 2 mg/kg BW midazolam + 2.5 mg/kg BW butorphanol.
It has previously been reported that mean BALF total protein concentration increased after anesthesia with the multiple-drug combination 0.375 mg/kg medetomidine, 2 mg/kg midazolam, and 2.5 mg/kg butorphanol (MMB2 in the present study); those authors concluded that this drug combination was unsuitable for use when examining BALF parameters (Senoh et al., 2016). The reported results are consistent with our results, and we agree with the conclusion about MMB2. MMB1 induced a milder increase in mean BALF albumin concentration compared with MMB2, and the other BALF parameters were comparable in rats given pentobarbital and isoflurane, regardless of whether or not the rats had received NiO intratracheally (i.e., regardless of whether or not they had acute pulmonary inflammation). Also, blood chemical parameters examined (except for mean serum glucose concentration) in untreated control rats given MMB1 were comparable in those given pentobarbital and isoflurane. Furthermore, a significant increase in mean BALF albumin in rats instilled with NiO compared with rats of vehicle control was detected as a pulmonary inflammatory response when MMB1 was administered. This suggests that this anesthetic combination with less medetomidine may be used when examining pulmonary inflammation via changes in BALF parameters, although the results of BALF albumin and serum glucose obtained were not suited to comparison with those obtained under pentobarbital or isoflurane.

In terms of stress response, it was reported that i.p. injection of 105 mg/kg BW of pentobarbital or saline raised plasma corticosterone level but 4% of isoflurane inhalation for 1 to 2 min did not influence it (Wu et al., 2015). Although whether i.p. injection of the anesthetic mixture of medetomidine–midazolam–butorphanol alters plasma corticosteron level or not has not been clear, an i.p. injection is considered to give more stress to rats compared with inhalation regardless of presence or absence of anesthetics. Thus, isoflurane inhalation might be also superior to i.p. injection of pentobarbital and the anesthetic mixture of medetomidine–midazolam–butorphanol from an ethical viewpoint of stress response.

Our results suggest that, of the anesthetics examined, isoflurane remains the most suitable when examining differences in BALF and blood chemistry parameters as part of toxicity studies in rats. The anesthetic mixture of medetomidine–midazolam–butorphanol can be used; however, when comparing data obtained with this combination with those obtained with pentobarbital or isoflurane, it is likely that BALF albumin concentrations and serum glucose concentration have been increased by the use of the combination anesthetic.

Conflict of interest---- This study was conducted under the Development of Innovative Methodology for Safety Assessment of Industrial Nanomaterials project, which is supported by the Ministry of Economy, Trade and Industry (METI) of Japan.

REFERENCES


