Comparative Medicine
Copyright 2005
by the American Association for Laboratory Animal Science

Vol 55, No 3
June 2005
Pages 256-264

Evaluation of Two Combinations of Domitor, Zoletil 100, and Euthatal to Obtain Long-term Nonrecovery Anesthesia in Sprague-Dawley Rats

Livia Ferrari,[1,*] Giuliano Turrini,[1] Chiara Rostello,[1] Alessia Guidi,[1] Alessandro Casartelli,[2] Alessandro Piaia, DVM,[2] and Matteo Sartori

We sought to evaluate a new protocol designed to maintain long-term, nonrecovery, surgical anesthesia in Sprague-Dawley rats. In the first phase, two groups of rats were anesthetized with two different dose combinations of Domitor (medetomidine) and Zoletil 100 (tiletamine–zolazepam) to investigate their efficacy in induction of anesthesia. One combination comprised Domitor at 35 μg/kg and Zoletil 100 at 40 mg/kg, whereas the other comprised Domitor at 50 μg/kg and Zoletil 100 at 20 mg/kg. Both combinations effectively induced deep anesthesia and caused no mortality, but the duration of anesthesia differed statistically. In the second phase, we induced anesthesia with both Domitor–Zoletil 100 dose combinations then investigated the possibility of maintaining anesthesia for 5 h by administering Euthatal (pentobarbitone) intra-arterially at 10 mg/kg hourly. Depth of anesthesia, mortality, physiological parameters, blood gas analysis, hematology, clinical chemistry, and postmortem histopathology were recorded. Euthatal provided stable long-term anesthesia with both dose combinations of Domitor–Zoletil 100. Seven of 8 (88%) animals anesthetized with Domitor at 50 μg/kg and Zoletil 100 at 20 mg/kg successfully were maintained under deep anesthesia for 5 h. Higher mortality (36% versus 12%) occurred in group of animals treated with Domitor at 35 μg/kg and Zoletil 100 at 40 mg/kg. This difference may be linked to dose-related respiratory depression, as alterations of arterial gas levels were noted. Our findings suggest that, when long-term nonrecovery anesthesia is required, doses of 50 μg/kg Domitor and 20 mg/kg Zoletil 100 are preferable when given with Euthatal to maintain physiological conditions in animals.

The 1:1 (wt/wt) combination of tiletamine hydrochloride and zolazepam hydrochloride is a nonopioid, nonbarbiturate injectable anesthetic initially approved for use in the United States under the commercial name of Telazol (A. H. Robins, Richmond, Va.) as an anesthetic and immobilizing agent in cats and dogs (17, 30). Tiletamine produces dissociative anesthesia, a state characterized by electroencephalographic evidence of dissociation of the thalamus and limbic system. Tiletamine is structurally similar to ketamine but has higher potency and duration of action. When used alone, tiletamine produces deep analgesia and a cataleptoid state, while maintaining normal pharyngeal–laryngeal reflexes. The treated animal's eyes remain open, with a slow nistagmic gaze (28).

Zolazepam is a benzodiazepine derivative, licensed by the Food and Drug Administration for use only in combination with tiletamine. Similar to other drugs of its class, it produces a tranquillizer effect with minimal depression of cardiorespiratory function. It has a rapid onset and short duration of action but lacks analgesic effects (22).

The two drugs, combined in a 1:1 ratio, offer a wide margin of safety and several pharmacological advantages, including rapid induction time, excellent muscle relaxation, and smooth recovery. Dosages of 20 to 40 mg/kg intraperitoneally (i.p.) produce anesthesia in Sprague-Dawley and Fischer 344 rats (21, 24, 26). However, the drug combination's main disadvantages are: variable analgesia, lengthy recovery period, and the absence of reversibility due to lack of an effective antidote. In addition, judging the depth of tiletamine–zolazepam anesthesia is difficult because the corneal, pedal, and swallowing reflexes remain intact.

To overcome some of these problems and to develop an anesthetic protocol suitable for long-term anesthesia in Sprague-Dawley rats for nonrecovery surgical models, we investigated combinations of medetomidine, zolazepam, and tiletamine (Domitor–Zoletil 100). We chose medetomidine because it is a potent α_2 agonist, similar to xylazine, but is much more specific and with a lower incidence of side effects (1, 19, 23). It can be used in many species to provide deep sedation and analgesia and can be rapidly and completely reversed using the specific α_2 antagonist atipamezole (13). The combination of medetomidine, tiletamine, and zolazepam has already been used successfully in wild animals (8-10), but there are no reports regarding its application in rats.

In the first part of our assessment (Study 1), we induced and maintained surgical anesthesia by two different dose combinations of Domitor–Zoletil 100 and evaluated their capacity to induce and support deep anesthesia. Immobilization and sleeping times were recorded.

Pentobarbitone has been widely used as laboratory animal anesthetic. When used alone, it is a poor analgesic and can cause severe cardiovascular and respiratory depression with a low safety margin (13). Nevertheless, from our experience, these side effects can be reduced using a decreased dose of pentobarbitone in combination with other anesthetics. We therefore undertook a study to determine whether additional slow infusion of pentobarbitone to Domitor–Zoletil 100 could provide long-term (5 h)

Received: 10/13/04. Revision requested: 2/11/05. Accepted: 2/15/05.
[1]Laboratory Animal Science Department, GlaxoSmithKline Medicine, Research Centre, Via Fleming 4, 37135, Verona, Italy; [2]Pathology Department, GlaxoSmithKline Medicine Research Centre, Via Fleming 4, 37135, Verona, Italy.
[*]Corresponding author.

Table 1. Protocols used for induction (Study 1) and maintenance (Study 2) of long-term anesthesia

Anesthetic protocol	Compound	Concentration (mg/ml)	Dose (mg/kg)	Dose volume (ml/kg)	Route
A	Domitor	0.7	0.035	0.05	i.m.
	Zoletil 100	20	40	2	i.p.
B	Domitor	1	0.05	0.05	i.m.
	Zoletil 100	10	20	2	i.p.
A + C	Domitor	0.7	0.035	0.05	i.m.
	Zoletil 100	20	40	2	i.p.
	Euthatal	6.25	10/h	1.6/h	i.a.
B + C	Domitor	1	0.05	0.05	i.m.
	Zoletil 100	10	20	2	i.p.
	Euthatal	6.25	10/h	1.6/h	i.a.

Working concentrations of Domitor and Zoletil 100 were obtained by diluting the commercially available solution with 0.9% sodium chloride solution. The working concentration of Euthatal was obtained by diluting the commercially available solution with 0.9% sodium chloride solution containing 10 IU/ml heparin.

anesthesia without the deleterious effects generally associated with pentobarbitone.

Materials and Methods

Animals and housing conditions. Specific pathogen-free male Sprague-Dawley rats from Charles River Italia (Calco, Lecco, Italy) were used. On the basis of quarterly rodent health surveillance by use of a sentinel program, this colony was verified to be free of all viral, bacterial, and parasitic pathogens as recommended by the Federation of European Laboratory Animal Science Association (20). This species, strain, and sex were selected as they are typical choices for preclinical and pharmacological studies.

Body weight of the rats ranged from 260 to 373 g and approximate age was 10 weeks on the day of the experiment. The animals were housed in stable groups of five rats each in polycarbonate cages with sawdust litter. During the pre-experimental period, Altromin R diet (Rieper, Vandoies, Italy) and filtered water from the normal domestic supply were provided ad libitum. Food was withheld 16 h before surgery, but water was supplied ad libitum.

Animals were maintained on a 12:12-h light:dark cycle (lights on, 6 a.m. to 6 p.m.). The room temperature was 20 to 22°C, and relative humidity was 45 to 65%. After arrival, animals were acclimated for at least 5 days before being used in the experiments.

All the experiments were carried out in accordance with Italian regulation governing animal welfare and protection (which is acknowledged in the European Directive 86/609/EEC) and according to internal review by the GlaxoSmithKline Committee on Animal Research & Ethics.

Anesthetics. The anesthetics used were: Domitor (1 mg/ml medetomidine, Farmos, Turku, Finland); Zoletil 100 (a combination of 50 mg/ml tiletamine and 50 mg/ml zolazepam, Virbac, Milano, Italy); and Euthatal (200 mg/ml pentobarbitone, Merial, Harlow, United Kingdom; containing). Domitor and Euthatal were kept at room temperature; Zoletil 100 was stored, after reconstitution of the powder, at 4°C and used within 3 days.

Anesthetic protocols. Details of the anesthetic protocols are given in Table 1. Domitor and Zoletil 100 were diluted in 0.9% sodium chloride solution and administered intramuscularly (i.m.; 0.05 ml/kg) and intraperitoneally (i.p.; 2 ml/kg), respectively. For protocol A, Domitor was diluted to 0.7 mg/ml and administered at a dose level of 35 µg/kg, and Zoletil 100 was diluted to 20 mg/ml and administered at 40 mg/kg. For protocol B, Domitor was diluted to 1 mg/ml and administered at a dose level of 50 µg/kg, and Zoletil 100 was diluted to 10 mg/ml and administered at 20 mg/kg. Euthatal was diluted to 6.25 mg/ml with saline containing 10 IU/ml heparin (Liquemin, Roche, Milano, Italy) and administrated intra-arterially (i.a.) at 10 mg/kg hourly (corresponding to 1.6 ml/kg hourly).

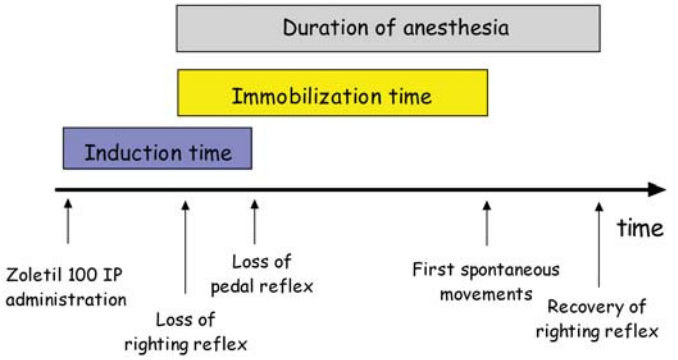

Figure 1. Time-related parameters of anesthesia recorded in Study 1.

Study 1: evaluation of efficacy, depth, and time of anesthesia induced by Domitor–Zoletil 100. (i) Administration of anesthetics. Animals were assigned randomly to two groups and serially numbered. Each animal was injected i.m. with Domitor and, after sedation occurred (usually within 15 min), Zoletil 100 was administered i.p. Six animals each were treated with protocols A and B (Table 1).

After injection of Zoletil 100, animals were returned to their home cages, and these placed on a water-bath heated plate (38°C). After the loss of the righting reflex, animals were laid in the dorsal recumbency position, breathing room air for the duration of the experiment. When the righting reflex returned, the animals first righted themselves, then began to walk. At that time, the animals were observed for at least one additional hour to record any abnormal behavior.

(ii) Depth of anesthesia. We assessed reflexes to monitor depth of anesthesia at 5 min after Zoletil 100 administration and then every 15 min until the animal's righting reflex returned. For the righting reflex, any attempt to move from dorsal to sternal recumbency was judged as a positive reflex response. Responses to painful stimuli were assessed using pedal withdrawal, palpebral, tail pinch, and cutaneous reflexes. For the pedal withdrawal reflex, the animal's hindlimb was extended slightly and the interdigital webbing of the foot firmly pinched using atraumatic forceps. A clear attempt to withdraw the limb was judged as a positive reaction (2, 14, 25). The palpebral reflex was assessed by light stimulation at the edge of the eyelid, by using atraumatic forceps. If blinking occurred, the response to stimuli was recorded as positive (16). For the tail pinch reflex, the operator used his/her fingers to pinch the rat's tail in the third distal quarter of the length of the tail. Any response was judged positive. For determination of cutaneous reflex, the skin was pinched in the ventral abdominal region by using sharp forceps. Any response was judged positive.

(iii) Time-related parameters of anesthesia (Fig. 1). Induction time was considered as the time from injection of Zoletil 100 until loss of the pedal withdrawal reflex**.** Immobilization

(time during which the animal makes no movements) was defined as the time span between loss of righting reflex and return of spontaneous movements. Duration of anesthesia was the time span which the animal showed no righting reflex (i.e., the time interval in which the animal is lying in the dorsal recumbency, occasionally showing movements or signs of arousal, but unable to right itself or walk).

Study 2: evaluation of the long-term anesthesia induced by Domitor–Zoletil 100 and maintained by Euthatal. The general design of this study consisted of anesthetizing two groups of animals with the two previously tested dose combinations of Domitor–Zoletil 100. Surgical catheterization of the carotid artery and jugular vein were performed for administration of anesthesia and sampling of blood, respectively. Moreover, a sham abdominal surgery was performed to simulate conditions under which the anesthesia protocols might be used. After cannulation anesthesia was supplemented with i.a. infusion of Euthatal for 5 h postsurgery through the same catheter used to monitor cardiovascular parameters. To avoid administration of excessive fluid volume to the rats, thus causing alteration of the cardiovascular parameters, a slow continuous infusion of Euthatal was used; this solution also included heparin to prevent clotting.

Anesthetic induction time, depth of anesthesia, mortality, and several physiological parameters (respiratory rate, blood pressure, body temperature, heart rate, blood gas analysis, hematology, and clinical chemistry) were evaluated for a period of 5 h postsurgery. Major organs were removed from all the animals and submitted for histopathology.

(i) Administration of anesthetics. Two groups of animals were anesthetized: one group (11 animals) with protocol A and the other (8 animals) with protocol B. After catheterization of the carotid artery, anesthesia in both groups was maintained with continuous i.a. infusion of 10 mg/kg hourly of Euthatal (protocol C, Table 1).

(ii) Surgical preparation of animals. After induction of anesthesia, the neck and abdomen were shaved and the skin disinfected with alcohol. The animals then were placed on interactive heating pads, connected to rectal probes.

(a) Tracheotomy. A midline skin incision was made along the length of the neck and, after separating the two halves of the stern hyoid muscle, the trachea was exposed. This was incised in the sublaryngeal region and a PE200 cannula was inserted into it and secured with a suture (2-0 silk, Johnson & Johnson International, Brussels, Belgium).

(b) Carotid artery cannulation. The right or left carotid artery was isolated and cannulated with a polyethylene catheter (PE50) filled with a heparinized solution of Euthatal, and then connected to an infusion pump and to a piezoelectric pressure transducer (Hewlett Packard). The position of the catheter was adjusted to obtain a clear pressure signal on the data acquisition system. The catheter was tied in place by two sutures (4-0 silk, Johnson & Johnson).

(c) Right jugular vein cannulation. The right jugular vein was isolated, and a polyvinyl chloride catheter, filled with heparinized saline solution, was inserted with the tip in the superior vena cava and tied in place by two suturing threads. The catheter was used to collect venous blood samples for hematology and clinical chemistry.

(d) Sham abdominal surgery. A midline incision was made on the abdominal skin and wall, along linea alba, to mimic the abdominal surgery that can take place during long-term nonrecovery anesthesia. These incisions then were closed using 3-0 silk or metal clips.

(iii) Indicators for depth of anesthesia. Depth of anesthesia was checked 5 min after Zoletil 100 administration, at the end of surgical preparation (30 min later), and then every 30 min for 5 h postsurgery. Pedal withdrawal and palpebral reflexes were checked on each animal (see Study 1).

(iv) Physiologic measurements. Respiratory rate (RR, breaths/min) was determined 10 min after the end of the surgery (t = 0 h) and then hourly for 5 h. Body temperature (T, °C) was monitored continuously through a rectal probe and recorded every 30 min. For each animal, systolic, diastolic, mean arterial blood pressure (BP, mm Hg) and heart rate (HR, beats/min [bpm]) were recorded continuously by using the Bioreport data acquisition system (Modular Instruments, Malvern, Pa.). Arterial blood samples were collected through the carotid catheter at the end of surgical preparation, at t = –0.17 h (10 min before t = 0 h), and at t = 5 h in order to determine oxygen partial pressure (PaO_2), carbon dioxide partial pressure ($PaCO_2$), and acid–base balance (pH value). Measurements were determined using a blood gas analyzer (AVL OPTI CCA Critical Care Analyzer, Roswell, N.M.).

The hematological parameters investigated were total leukocyte count (WBC), erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), mean platelet volume (MPV), neutrophil count (NEUT), lymphocyte count (LYM), monocyte count (MON), eosinophil count (EOS), basophil count (BAS), and reticolocyte count (RET). The clinical chemistry parameters investigated were Ca, P, Na, K, Cl, glucose (GLU), total protein (TP), creatinine (CRE), albumin (ALBM), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamic dehydrogenase (GLDH), blood urea nitrogen (BUN), cholesterol (CHOL), bilirubin (BIL), and triglycerides (TRI). Two days before surgery (through a needle inserted in the tail vein) and at 0 and 5 h postsurgery (through the catheter inserted in the jugular vein), 1 ml of blood was collected for determination of various hematological and clinical chemistry parameters.

After collection, blood samples were placed into appropriate vials containing tripotassium ethylenediamine tetra-acetate for hematological measurements and without anticoagulant for clinical chemistry analysis on serum. Hematological analyses were performed using an ADVIA 120 Bayer (Milan, Italy) analyzer with veterinary software, and clinical chemistry parameters were determined using a Hitachi 912 analyzer (Milan, Italy).

Euthanasia. Euthanasia was carried out at t = 5 h by injection of pentobarbitone (200 mg/kg) into the carotid artery (4, 11, 12).

Post mortem examination and histopathology. The following tissues were placed in 10% buffered formalin: brain, heart, lungs, trachea, liver, and kidneys. All tissues were processed using routine histological procedures, stained with hematoxylin and eosin, and examined microscopically.

Data analysis. All the experimental data were analyzed using the Excel software program (Microsoft, Redmond, Wash.) and are summarized as mean and standard deviation (SD). Statistical comparison between groups of animals anesthetized with different protocols was performed with Dunnett's multiple com-

Table 2. Immobilization and duration of anesthesia and of loss of reflexes (in h; mean ± SD; n = 6 per protocol) in animals anesthetized with protocols A and B during Study 1

	A	B
Immobilization time	2.61 ± 0.08	2.37 ± 0.29
Sleeping time*	3.35 ± 0.34	2.83 ± 0.31
Pedal withdrawal reflex*	2.04 ± 0.12	1.71 ± 0.07
Palpebral reflex	1.96 ± 0.29	2.09 ± 0.13
Tail pinch reflex	2.87 ± 0.02	2.80 ± 0.16
Cutaneous reflex	3.15 ± 0.34	2.80 ± 0.32

*, Statistically significant difference ($P < 0.05$) between A and B.

parison test. Differences were considered significant when the P value was < 0.05.

Results

Study 1: evaluation of efficacy, depth, and time of the anesthesia induced by Domitor\Zoletil 100. On average, animals lost their righting reflex 4 min after i.p. injection of Zoletil 100, independently of the protocol used. Pedal, palpebral, tail, and cutaneous reflexes were all negative at the first check (5 min after injection of Zoletil 100), indicating that a surgical level of anesthesia was achieved. Table 2 reports the immobilization and duration of anesthesia and of loss of reflexes in rats anesthetized with the two combinations of Domitor–Zoletil 100. Animals treated with protocols A and B did not show any spontaneous movement for 2.61 ± 0.08 h and 2.37 ± 0.29 h, respectively, and they were again awake and moving after 3.35 ± 0.34 h and 2.83 ± 0.31 h, respectively, showing a Zoletil 100 dose-dependency trend. All rats in both groups survived, and no abnormal behavior was recorded during the observation period.

Depth and quality of anesthesia were determined by use of reflex tests. The most sensitive indicators for surgical tolerance were pedal withdrawal and palpebral reflexes, which were the first reflexes recovered. Animals anesthetized with protocols A and B recovered the pedal reflex after 1.71 ± 0.07 h and 2.04 ± 0.12 h, respectively, and the palpebral reflex after 1.96 ± 0.29 h and 2.09 ± 0.13 h, respectively. In general, no tail or cutaneous reflexes were detected until the end of the duration of anesthesia in both groups of animals.

Study 2: evaluation of the long-term anesthesia induced by Domitor–Zoletil 100 and maintained by Euthatal. (i) Efficacy, depth of anesthesia, and mortality. As described for Study 1, both protocols successfully induced and maintained anesthesia. All treated animals lost the righting reflex within 5 min after i.p. injection of Zoletil 100. As a consequence of observations made in Study 1, only pedal withdrawal and palpebral reflexes were tested to verify the depth of anesthesia. No response to painful stimuli was recorded at the first check, indicating that surgical anesthesia was achieved within 6 min after Zoletil 100 injection and was maintained for long period in both protocols.

Seven rats per protocol were maintained successfully under anesthesia for 5 h; therefore mortality for protocol A + C was 36% (4 of 11) and 12% (1 of 8) for protocol B + C. Animals died within 2 h of the observation period for protocol A + C and within 3.5 h for protocol B + C.

(ii) Physiological parameters. Marked respiratory depression was recorded for animals anesthetized with protocol A + C during the initial phase of the anesthesia. At t = 0, RR was 43 ± 8 breaths/min (Fig. 2A), compared with the reference value of 97 ± 2 breaths/min reported for conscious rats of the same strain (5). Despite a steady increase during the observation period, final values were far from physiological conditions. A similar situation occurred for animals anesthetized with protocol B + C but respiratory depression was less marked: RR was 58 ± 8 breaths/min at t = 0 h and increased to 78 ± 12 breaths/min at the end of the observation period (20 breaths/min higher than that for protocol A + C).

Figure 2B reports the body temperature in rats during the observation period (5 h). In both groups, hypothermia was recorded a t = 0 h, with rectal temperatures of 33.9 ± 1.1°C for protocol A + C and 34.6 ± 1.1°C for protocol B + C. Animals in group B + C recovered physiological body temperature (~37.5°C) faster than animals in group A + C, after 1.5 h of monitoring of anesthesia.

Direct comparison of the mean blood pressures (MBP) of animals anesthetized with the two anesthetic protocols is reported in Fig. 2C. No statistical differences were observed between the two groups for 4 h postsurgery, when the MBP ranged from 70 to 80 mm Hg. After this period, the MBP of rats anesthetized with protocol B + C increased to values similar to those reported in the literature for conscious rats of the weight range used (250 to 300 g, 90 ± 2 mm Hg [3]; 361 ± 7 g, 103 ± 6 mm Hg [6, 7]), whereas animals under anesthesia from protocol A + C maintained a hypotensive state.

Figure 2D shows HR recorded during the evaluation of the two anesthetic protocols. The same cardiac depression (no statistical differences) was observed in both groups during the first hour of anesthesia. However, during the following 4 h, animals anesthetized with protocol B + C achieved physiological conditions faster than did those anesthetized with protocol A + C. At t = 5 h, mean HR was 270 bpm for protocol A + C and 310 bpm for protocol B + C, both of which values are very close to those reported in the literature for conscious rats of the same weight (200 to 300 g, 332 bpm; 250 to 300 g, 242 ± 17 bpm) (3, 5).

Figure 3 shows the PaO_2, $PaCO_2$, and pH values at the end of surgical preparation (t = –0.17 h) and at t = 5 h. PaO_2 of all anesthetized rats (Fig. 3A) was below the physiologic range (70 to 110 mm Hg) at the first time point, without statistical difference between the two groups. At t = 5 h, mean PaO_2 for group A + C had decreased to 42 mmHg, whereas that of group B + C (75 mm Hg) had returned to physiological levels.

Blood $PaCO_2$ (Fig. 3B) remained above the physiological range (35 to 45 mmHg) for all animals anesthetized with protocol A + C at both time points, thus causing a blood pH lower than the physiological value of 7.4 (Fig. 3C). A statistically different situation was noted in animals anesthetized with protocol B + C: for this regimen, only one deviation from the reference range was noted (one rat at t = –0.17 h). Consequently, with the exception of one rat, arterial pH of all animals treated with protocol B + C was within the physiological range (7.4 to 7.6) at t = –0.17 h and t = 5 h.

Individual tables and summary graphs of noteworthy hematological parameters are reported in Fig. 4. No substantial differences between the groups of animals were observed. No relevant abnormalities were detected 10 min after the end of surgery (t = 0 h). However in all animals at t = 5 h, NEUT was increased by a factor of 2 to 4 and LYM was decreased to 1/3 to 1/5 of the basal value; these abnormalities could be related to inflammation processes consequent to surgical procedures. No significant change was noted in any other parameter measured (data not reported),

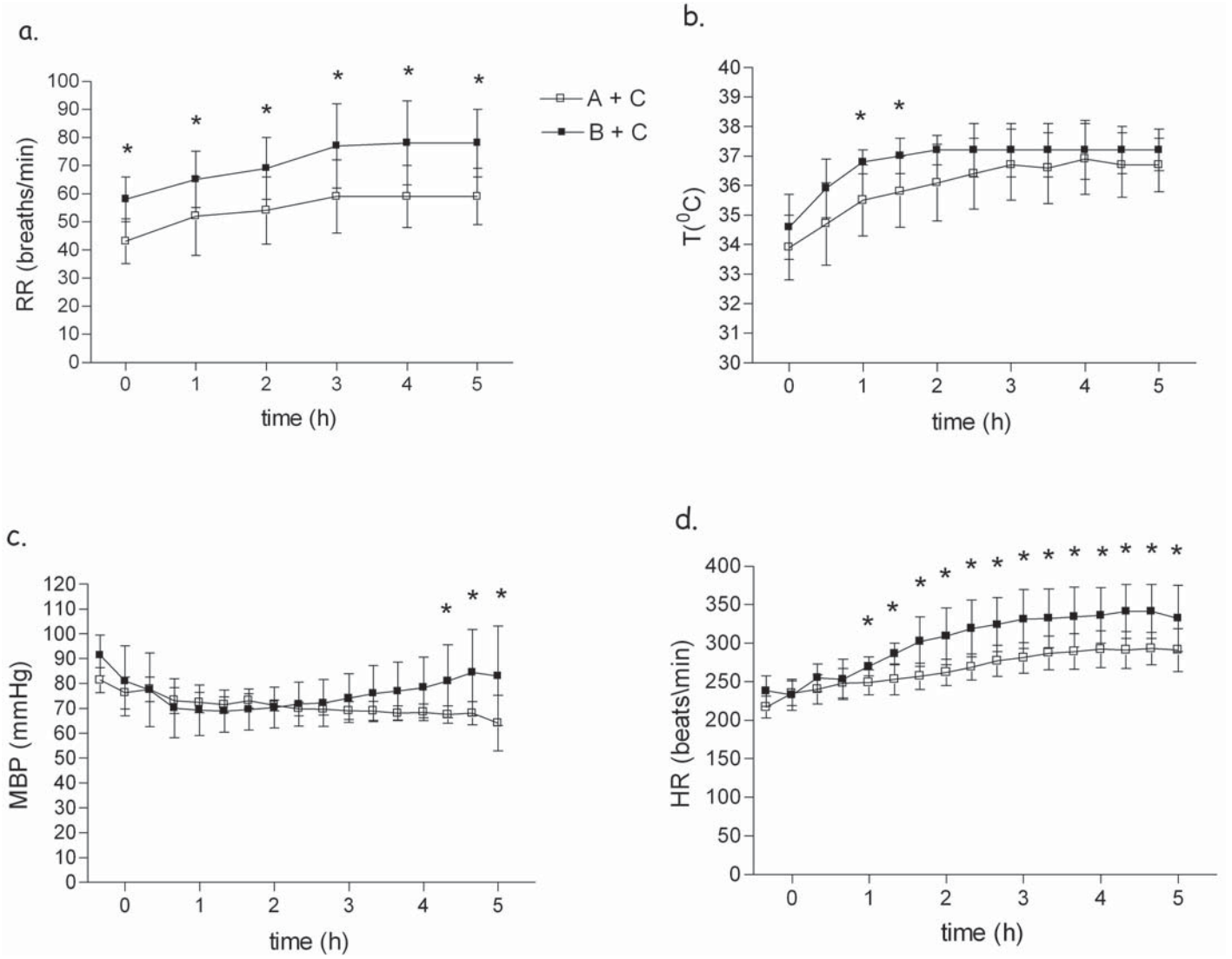

Figure 2. Values (mean ± SD; n = 7 per protocol) for (a) respiratory rate (RR), (b) body temperature (T), (c) mean blood pressure (MBP), and (d) heart rate (HR) during the observation period (5 h) in rats anesthetized with protocols A + C (open circles) and B + C (solid circles). *, Statistical difference ($P < 0.05$) between A + C and B + C.

and individual values generally remained within normal ranges.

Individual tables and summary graphs of noteworthy clinical chemistry parameters are reported in Fig. 5. As for hematology, no substantial differences between the two groups were observed. All anesthetized animals displayed a constant trend toward decreased serum ALBM and TP at all postsurgery time points. The decrease in ALBM could be related to a reduced ability of the liver to synthesize this protein. Na and K changes could be due to slight decreases in renal water adsorption. This theory is supported by the autopsy macroscopic examination, when the animals' bladders were full of urine. Moreover, K might have been involved in the compensatory mechanism in response to the acidosis due to respiratory depression, and increased clearance of this ion through kidneys can be hypothesized. All other parameters remained within physiological ranges (data not reported).

Post mortem examination: histopathology. Only minimal histopathological changes related to long-term intravenous anesthesia were noted in the liver and lungs of rats anesthetized with protocol A + C and the liver of rats anesthetized with protocol B + C.

In four of the eight animals anesthetized with protocol A + C, the liver showed very slight hepatocellular vacuolation (multifocal to diffuse). Histologically this change consisted of fine clear vacuoles distributed within the cytoplasm, mainly in the centrilobular hepatocytes. In addition, alveolar distension was seen in two of eight rats, in which the alveoli appeared larger than normal, with no rupture of the wall.

In animals anesthetized with protocol B + C, only one animal showed very slight multifocal hepatocellular vacuolation.In addition, multifocal acute necrosis, characterized by foci of coagulative necrosis mixed with hemorrahage distributed mainly centrilobularly, was seen in another animal.

Discussion

In this study we investigated new dose combinations of Domitor (medetomidine) and Zoletil 100 (tiletamine and zolazepam) to obtain deep surgical anesthesia and, with the addition of slow infusion of Euthatal, stable long-term anesthesia in Sprague-

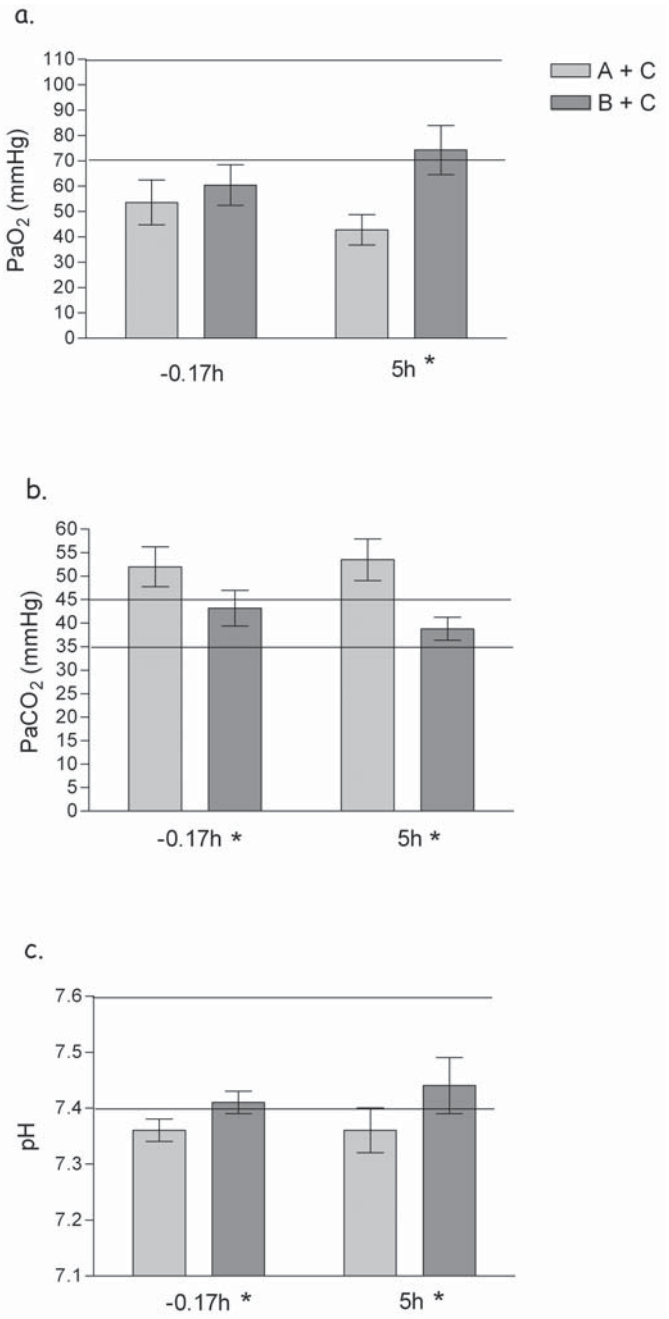

Figure 3. Values (mean ± SD; n = 7 per protocol) for (a) oxygen partial pressure (PaO_2), (b) carbon dioxide partial pressure ($PaCO_2$), and (c) pH during the observation period (5 h) in rats anesthetized with protocols A + C and B + C. The lines indicate values within the physiological range. *, Statistical difference ($P < 0.05$) between A + C and B + C at each time point.

Dawley male rats.

Both of the anesthetic protocols investigated (A + C and B + C) provided stable surgical anesthesia for more than 5 h, with rapid onset of anesthetic effect. However, comparison of the mortality data, physiological parameters, arterial gas values, hematological and clinical chemistry parameters, and histopathological outcomes revealed that protocol B + C provided a safer and more physiological outcome.

The decreased mortality seen with protocol B + C could be due to a less marked respiratory depression than that associated with protocol A + C. Severe dose-related respiratory depression has been reported to be one of the most troublesome effects of pentobarbitone in rats (13, 29). Nevertheless, the respiratory depression we saw more likely was due to Zoletil 100, as animals were starting to recover from the respiratory depression during the last 2 h of anesthesia, when the effect of the Domitor–Zoletil 100 combination likely was wearing off. Zoletil 100 is known to cause respiratory depression in rats (27). In particular, this effect may be caused by tiletamine which, similarly to ketamine, induces dose-dependent hypoventilation and apnea in animals (17). These findings suggest that mortality of animals anesthetized with protocol A + C could be decreased with artificial ventilation, although this possibility was not investigated in our study. Results of the blood gas analysis reflected the effects of respiratory depression. In animals anesthetized with protocol A + C the partial pressures of arterial gasses, and consequently pH, were outside physiological ranges during the observation period (5 h), whereas a more physiological picture was obtained throughout anesthesia in rats anesthetized with protocol B + C.

All anesthetized animals showed initial hypothermia, hypotension, and bradycardia. Hypothermia is a typical consequence of anesthesia of animals of small size. The surgical procedures performed might also lead to further decreased body temperature. Nevertheless, the body temperatures of animals placed on interactive heating pads had returned to basal levels by 1.5 h from the end of surgical procedures. The observed hypotensive effect is due to the combination of Domitor–Zoletil 100 and Euthatal. In particular, Domitor and Euthatal are known to cause hypotension in anesthetized rats (13).

The heart rate reported for both evaluated groups during the first hour can be considered to be at the lower end of physiological values reported for the rat (3, 5). The rise in heart rate observed after this period (particularly for animals anesthetized with protocol B + C) probably is due to a compensatory effect for the hypotension previously reported. Nevertheless, the extent of these effects (hypothermia, hypotension, and bradycardia) in animals anesthetized with protocol B + C was not as marked as in animals in the A + C group, and physiological values were recovered faster in the B + C group.

Hematological and clinical chemistry findings reflected the respiratory depression, the decrease in blood pressure, and possibly inflammatory processes caused by the surgical procedures. No other relevant alterations demonstrating toxicity affecting specific target organs were noted.

Histopathological changes in the liver (fine hepatocellular vacuolation) are consistent with acute cellular swelling, a reversible cell response to injury such as hypoxia (15). We noted acute necrosis in only one animal, which was treated with protocol B + C. Because centrilobular hepatocytes contain high levels of drug-metabolizing enzymes, reactive metabolites are liable to be formed in the centrilobular zone and cause tissue damage. For this reason, the possibility of an interaction of administered drugs (or their metabolites) can not be excluded. Both mechanisms may have had an effect on the liver of this animal. The observed histopathological findings in the lungs might be related to overinflation, which is an acute, minor, transient enlargement of airspaces that is known to occur after obstruction or diameter reduction of the airways (15).

Together, our findings suggest that combination of medetomi-

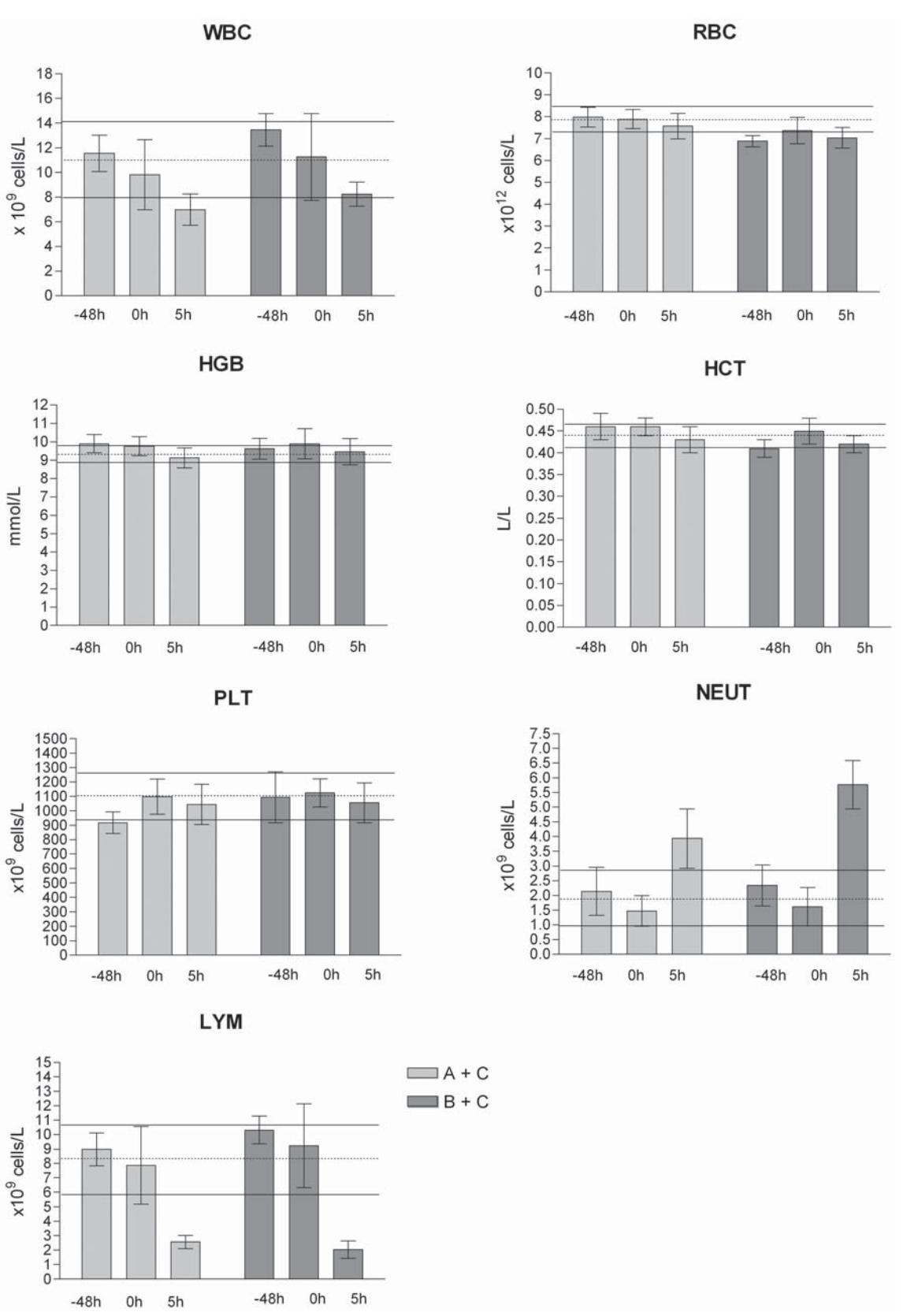

Figure 4. Hematological parameters (mean ± SD; n = 7 per protocol) of rats anesthetized with protocols A + C and B + C. Levels were measured 2 days before the study, 10 min after surgery, and after 5 h of anesthesia. Reported parameters are: total leucocyte count (WBC), erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), platelet count (PLT), neutrophil count (NEUT), and lymphocyte count (LYM). Solid lines indicate the range of normal values for conscious male Sprague-Dawley rats, and the dotted lines indicate mean values.

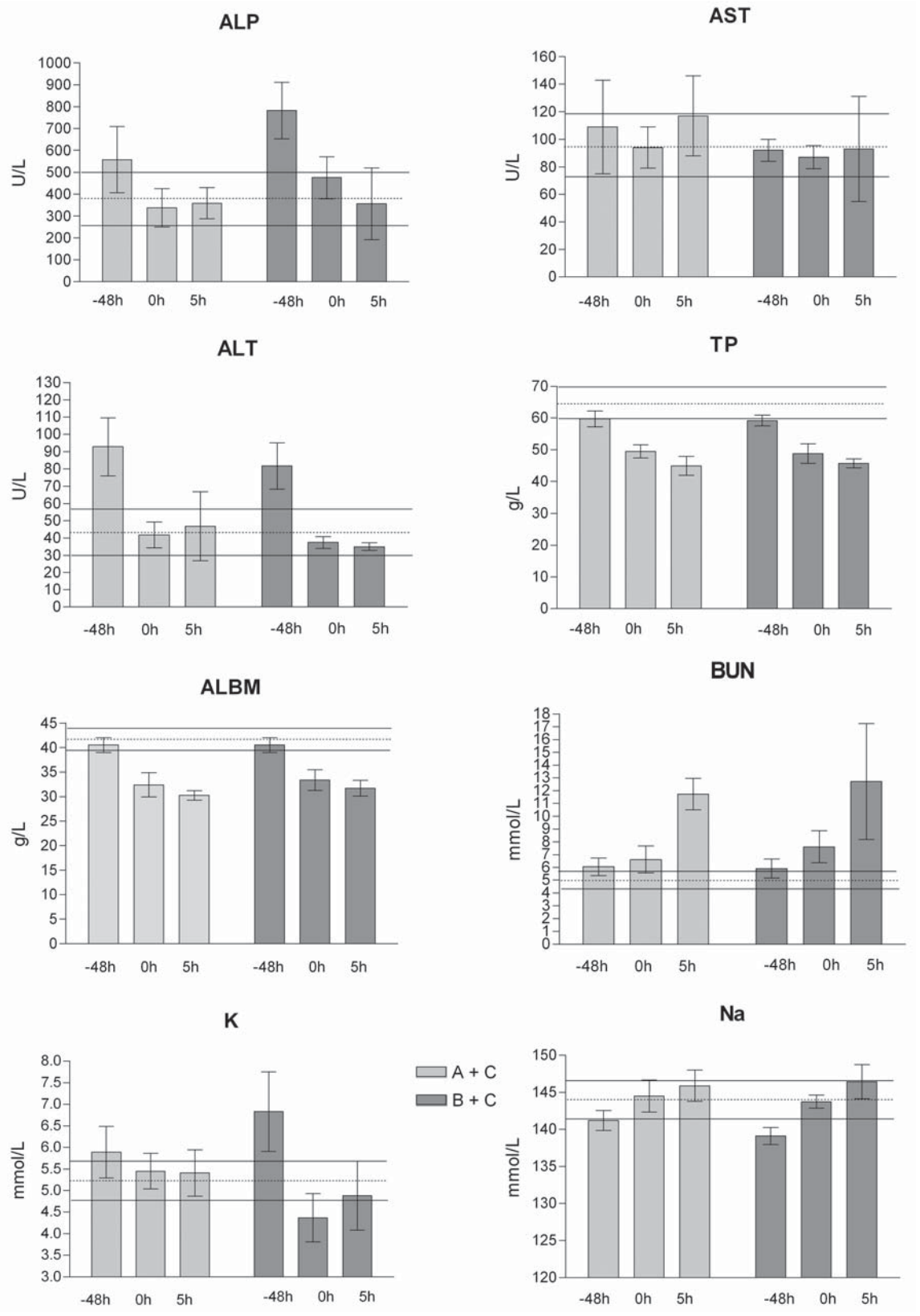

Figure 5. Clinical chemistry parameters of rats (mean ± SD; n = 7 per protocol) anesthetized with protocols A + C and B + C. Levels were measured 2 days before the study, 10 min after surgery, and after 5 h of anesthesia. Reported parameters are: alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), albumin (ALBM), blood urea nitrogen (BUN), Na, and K. Solid lines indicate the range of normal values for conscious male Sprague-Dawley rats, and the dotted lines indicate mean values.

dine, tiletamine, and zolazepam effectively induces anesthesia in Sprague Dawley rats and, with the addition of slowly infused Euthatal, it is possible to obtain long-term nonrecovery anesthesia. Respiratory depression is the leading side effect that we identified. As this respiratory depression is dependent on the Zoletil 100 dose, use of the protocol comprising 50 μg/ml Domitor, 20 mg/kg Zoletil 100, and 10 mg/kg/h Euthatal is preferable for studies requiring deep, long-term, nonrecovery anesthesia. This protocol also offers a low rate of mortality and minimal respiratory distress. To further improve this protocol, mechanical ventilation of animals should be investigated. Moreover, a practical advantage in the use of Domitor–Zoletil 100 is that neither agent is a controlled substance. Finally, the anesthesia produced by the association of Domitor–Zoletil 100 can be rapidly and completely reversed using the specific α_2 antagonist atipamezole (18, 19, 23). These characteristics prompt further interest in the use of tiletamine–zolazepam and medetomidine in combination and suggests their possible wider application (e.g., in recovery surgery).

References

1. **Aantaa, R. and M. Scheinin.** 1993. Alpha2-adrenergic agents in anaesthesia. Acta Anaesthesiol. Scand. **37:**433-448.
2. **Arras, M., P. Autenried, A. Rettich, D. Spaeni, and T. Rülicke.** 2001. Optimization of intraperitoneal injection anesthesia in mice: drugs, dosages, adverse effects, and anesthesia depth. Comp. Med. **51:**443-456.
3. **Barringer, D. L. and R. D. Bunag.** 1990. Differential anesthetic depression of chronotropic baroreflex in rats. J. Card. Pharmacol. **15:**10-15.
4. **Beaver B. V., W. Reed, S. Leary, B. McKiernan, F. Bain, R. Schultz, B. T. Bennett, P. Pascoe, E. Shull, L. C. Cork, R. Francis-Floyd, K. D. Amass, R. Johnson, R. H. Schmidt, W. Underwood, G. W. Thornton, and B. Kohn.** 2001. Report of the AVMA panel on euthanasia. J. Am. Vet. Med. Assoc. **218:**669-696.
5. **Brevard, M. E., T. Q. Duong, J. A. King, and C. F. Ferris.** 2003. Changes in MRI signal intensity during hypercapnic challenge under conscious and anesthetized conditions. Magn. Res. Imaging **21:**995-1001.
6. **Bunag, R. D. and L. W. Davidow.** 1996. Aging impairs heart rate reflexes earlier in female than in male Sprague-Dawley rats. Neurobiol. Aging **17(1):**87-93.
7. **Bunag, R. D., C. V. Thomas, and J. R. Mellick.** 2001. Ketanserin versus urapidil: age-related cardiovascular effects in conscious rats. Eur. J. Pharmacol. **435:**85-92.
8. **Cattet, M. R., N. A. Caulkett, S. C. Polischuk, and M. A. Ramsay.** 1997. Reversible immobilization of free-ranging polar bears with medetomidine-zolazepam-tiletamine and atipamezole. J. Wildl. Dis. **33(3):**611-617.
9. **Caulkett, N. A. and M. R. Cattet.** 1997. Physiological effects of medetomidine–zolazepam–tiletamine immobilization in black bears. J. Wildl. Dis. **33(3):**618-622.
10. **Caulkett, N. A., Cattet M. R., J. M. Caulkett, and S. C. Plischuk.** 1999. Comparative physiologic effects of telazol, medetomidine–ketamine, and medetomidine–telazol in captive polar bears. J. Zoo Wildl. Med. **30(4):**504-509.
11. **Close, B., K. Banister, V. Baumans, E. M. Bernoth, N. Bromage, J. Bunyan, W. Erhardt, P. Flecknell, N. Gregory, H. Hackbarth, D. Morton, and C. Warwick.** 1996. Recommendations for euthanasia of experimental animals: part 1. Lab Anim. **30:**293-316.
12. **Close, B, K. Banister, V. Baumans, E. M. Bernoth, N. Bromage, J. Bunyan, W. Erhardt, P. Flecknell, N. Gregory, H. Hackbarth, D. Morton, and C. Warwick.** 1997. Recommendations for euthanasia of experimental animals: part 2. Lab Anim. **31:**1-32.
13. **Flecknell, P. A.** 1996. Laboratory animal anaesthesia, 2nd ed. Academic Press, London.
14. **Gardner, D. J., J. A. Davis, P. J. Weina, and B. Theune.** 1995. Comparison of tribromoethanol, ketamine/acetylpromazine, telazol/xylazine, pentobarbital, and methoxyflurane anesthesia in HSD: ICR Mice. Lab. Anim. Sci. **45:**199-204.
15. **Jubb, K. V. F., P. C. Kennedy, and N. Palmer.** 1993. Pathology of domestic animals, 4th ed., vol. 2. Academic Press, London.
16. **Kohn, D. F., S. K. Wixson, and G. J. Benson.** 1997. Anaesthesia and analgesia in laboratory animals. Academic Press, London.
17. **Lin, H. C., J. C. Thurmon, G. J. Benson, and W. J. Tranquilli.** 1993. Telazol—a review of its pharmacology and use in veterinary medicine. J. Vet. Pharmacol. Ther. **16:**383-418.
18. **MacDonald, E., A. Haapalinna, R. Virtanen, and R. Lammintausta.** 1989. Effects of acute administration of medetomidine on the behaviour, temperature and turnover rates of brain biogenic amines in rodents and reversal of these effects by atipamezole. Acta Vet. Scand. Suppl. **85:**77-81.
19. **MacDonald, E. and R. Virtanen.** 1992. Review of the pharmacology of medetomidine and detomidine: two chemically similar α2-adrenoreceptor agonists used in veterinary sedatives, p. 181-191. In C. E. Short and A. Van Poznak (ed.), Animal pain. Churchill-Livingstone, New York.
20. **Nicklas, W., P. Baneux, R. Boot, T. Decelle, A. A. Deeny, M. Fumanelli, and B. Illgen-Wilcke.** 2002. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab Anim. **36:**20-42.
21. **Silvermann, J. and M. Bush.** 1983. Evaluation of a combination of tiletamine and zolazepam as an anaesthetic for laboratory animals. Lab. Anim. Sci. **33:**457-460.
22. **Stoelting, R. K.** 1987. Benzodiazepine, p.117-133. *In* R. K. Stoelting, Pharmacology and physiology in anaesthetic practice. J. B. Lippincott, Philadelphia.
23. **Virtanen, R.** 1989. Pharmacological profiles of medetomidine and its antagonist, atipamezole. Acta Vet. Scand. **4:**29-37.
24. **Ward, G. S., D. O. Johnson, and C. R. Roberts.** 1974. The use of CI-744 as an anesthetic for laboratory animals. Lab. Anim. Sci. **24:**737-742.
25. **Whelan, G. and P. A. Flecknell.** 1994. The use of etorphine/methotrimeprazine and midazolam as an anesthetic technique in laboratory rats and mice. Lab. Anim. **28:**70-77.
26. **Wilson, R. P., I. S. Zagon, and D. R. Larach.** 1992. Antinociceptive properties of tiletamine–zolazepam improved by addition of xylazine or butorphanol. Pharmacol. Biochem. Behav. **43:**1129-1133.
27. **Wilson, R. P., I. S. Zagon, D. R. Larach, and C. M. Lang.** 1992. Cardiovascular and respiratory effects of tiletamine–zolazepam. Pharmacol. Biochem. Behav. **44:**1-8.
28. **Winters, W. D., T. Ferrer-Allado, and C. Guzman-Flores.** 1972. The cataleptic state induced by ketamine: a review of the neuropharmacology of anesthesia. Neuropharmacology **11:**303-315.
29. **Wixson, S. K., W. J. White, H. C. Hughes, C. M. Lang, and W. K. Marshall.** 1987. The effects of pentobarbital, fentanyl–droperidol, ketamine–xylazine, and ketamine–diazepam on arterial blood pH, blood gases, mean arterial blood pressure, and heart rate in adult male rats. Lab. Anim. Sci. **37:**736-742.
30. **Wong, A. and S. M. Bandiera.** 1996. Inductive effect of Telazol on epatic expression of cytochrome P450 2B in rats. Biochem. Pharmacol. **52:**735-742.