Frequently applied ketamine, medetomidine and thiopental anaesthesia induces high mortality in Wistar rats

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Abstract. – **OBJECTIVE:** Fatal reactions to the combination of ketamine-medetomidine and thiopental in Wistar rats are described in two different models of orthodontic tooth movement.

MATERIALS AND METHODS: Thirty male rats were divided into two groups that required repeated anaesthesia during a 42-day study period, once a week or more frequently depending on the experimental group. The combination of ketamine [50 mg/kg body weight (b.w.)] and medetomidine (67 µg/kg b.w.) was administered intraperitoneally. Thiopental (8.3 mg/kg b.w.) was administered intraperitoneally 5 minutes later, barring any observable adverse reactions to the anaesthesia.

RESULTS: Twelve animals died, though none during the first two procedures. Three animals died shortly after the administration of a ketamine-medetomidine combination, and the remainder died 10-25 minutes later. Only four of the affected animals received thiopental before their death on a particular day. As ten rats died in the more frequently anaesthetized group, repeated anaesthesia was suspected to be the cause of the increased mortality.

CONCLUSIONS: Obstruction of the respiratory airways by saliva with subsequent suffocation may have been one of the causes of death, as it appeared in all the affected animals. Although the combination of ketamine and an alpha-2 adrenergic agonist is generally considered to be safe in rats, we propose that studies utilizing protocols requiring repeated anaesthesia adhere to a minimum safety period of 8.5 days between anaesthesia events. Alternative anaesthetic protocols should be employed if adherence to this is not possible due to the nature of the study.

Key Words:
Rat, Ketamine, Medetomidine, Thiopental, Serious adverse reaction.

Introduction

Ketamine was primarily developed as an animal and human anaesthetic which demonstrated a good safety profile of repeated anaesthesia in monkeys[1]. In the last decade, ketamine has been experiencing a renaissance of use as a novel antidepressant agent, an action clinically evaluated by Zarate et al[2] and utilized for ameliorating the symptoms of treatment-resistant depression in the form of a spray[3]. Despite its relatively favourable safety profile, the potential risks of repeated application in human use, as well as in animal anaesthesia, should be considered when developing ketamine as a rapid-acting antidepressant. Ketamine can induce adverse reactions, including cardiovascular events, which usually occur following high doses or prolonged treatment[4]. These cardiovascular adverse reactions can be attributed to the systemic release of catecholamines and the inhibition of norepinephrine reuptake in peripheral nerves and in non-neuronal tissues, such as the myocardium[5].

In laboratory animals, ketamine hydrochloride is used for the induction and maintenance of anaesthesia in rats – one of the most extensively studied experimental animals. Ketamine may be combined with various other drugs, including adrenergic alpha-2 agonists and barbiturates (e.g., thiopental)[6-9], with a combination of ketamine and an alpha-2 agonist being widely accepted for the anaesthesia of laboratory rodents[7,8]. One of the general uses of anaesthesia utilizing these substances is to prevent pain and unnecessary suffering during studies where anaesthetic agents are employed prior to the introduction of substances

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or procedures of interest. While these anaesthetic protocols are designed to produce as little influence on the study outcomes and results as possible, drugs and/or their combinations used in anaesthesia may still cause adverse reactions on their own and may interfere with the objectives of the study.

In rats, such adverse reactions can be observed when combinations of high doses of anaesthetic compounds are used, which may induce hypotension and respiratory depression[9,10] as well as a dose-dependent reduction in body temperature[11]. Ketamine may also induce hypersalivation[12]. While high doses of the alpha-2 agonist medetomidine may induce strong respiratory depression, doses ranging from 150 to 250 µg/kg were found to be sufficient for sedation in a study of rats where medetomidine was the sole agent, without the occurrence of respiratory depression[13].

Used concurrently, a combination of ketamine and medetomidine is generally regarded as a safe option in rats, even when repeated anaesthesia is required; an increase in anaesthetic mortality was previously attributed to compounds used in conjunction with the administered anaesthetics[14].

Of other adverse reactions, the combination of ketamine and another alpha-adrenergic agonist, xylazine, induced tissue damage after intramuscular injection in some strains of rats[8]. A combination of ketamine and medetomidine was shown to reduce such tissue damage and was thus chosen as the preferred combination for our 6-week study[15], as well as to avoid the excessive use of barbiturates in anaesthesia that would induce respiratory depression, hypotension[9,10], and profound hypothermia[11].

The aim of this study was to present, evaluate and explain the observed high mortality of Wistar rats after repeated anaesthesia consisting of ketamine, medetomidine and thiopental. A further goal was to define the cut-off values for a safe frequency of repetition of such a protocol of anaesthesia, based on this incidental, convenience set of data.

Materials and Methods

A group of 30 male Wistar rats was included in a study which required repeated, short-lasting anaesthetic procedures at weekly intervals. The experiment was carried out in accordance with permission issued by the Veterinary Administration of the Republic of Slovenia (No. 323-02-234/2005/2).

The animals used were obtained from the in-house breeding facility (Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia) and were clinically healthy. Their microbiological status was evaluated before the study commenced. The rats were housed in groups of five, in polycarbonate cages (Ehret, Wandlitz, Germany) with softwood bedding (Rettenmaier und Söhne, Rosenberg, Germany). They weighed from 310 to 350 g at the beginning of the study. The rats had unlimited access to drinking water and were fed a rodent diet (Krka d.d., Novo Mesto, Slovenia).

The rats were randomized into 2 groups of 15 animals. After the accommodation period, the rats were anaesthetized, and a super-elastic closed coil spring was placed between the first left maxillary molar and the incisors to induce orthodontic tooth force. The spring was attached to the molar by a stainless-steel ligature wire. In the first group of rats, the coil spring was attached to the incisors using a surgical steel wire, which was bent around the teeth. In the second group of rats, in addition to the bending of the surgical steel wire around the teeth, a hole was drilled through the approximal surfaces of the incisors, and the surgical steel wire was placed through the hole to improve fixation. To monitor tooth movement, weekly measurements of the distances between the teeth were made. The experimental time was set to 42 days. The animals were placed under general anaesthesia once weekly to monitor tooth movement. However, additional anaesthetic procedures in between measurements were required in the first group to reattach the coil spring, as it often detached from the teeth.

For general anaesthesia, the animals received an intraperitoneal injection of a combination of 50 mg/kg body weight (b.w.) ketamine ("Bioketan", Vetoquinol Zaklady Farmaceuticzne Biowet Gorzów, Gorzów Wielkopolski, Poland); and 67 µg/kg b.w. medetomidine ("Domitor", Pfizer Animal Health, Brussels, Belgium). Barring any adverse reactions, 8.3 mg/kg b.w. thiopental ("Tiopental", Pliva, Zagreb, Croatia) was administered, also intraperitoneally, 5 minutes after the ketamine-medetomidine.

In the preliminary experiments, no thiopental was used. However, spontaneous contractions of the masticator muscles during ketamine-medetomidine anaesthesia made precise measurements impossible. Thiopental was therefore administered in order to eliminate these contractions. A brief clinical examination of the animals (auscultation of the respiratory and cardiovascular sys-

tem, examination of the fur and visible mucosa, and palpation of the abdominal cavity) was carried out prior to the procedure (to ensure that the animals were healthy enough to safely undergo anaesthesia). After the procedure, the animals were closely observed until full recovery. No antibiotic nor analgesic postoperative therapy was necessary, as the dental procedure neither injured the surrounding tissues nor induced massive or painful trauma.

The data obtained from the survival of the rats were visualized and analysed with the use of SPSS v.25 (IBM, Armonk, NY, USA), MATLAB R2019b (MathWorks, Massachusetts, MA, USA) and PRISM 9 (GraphPad, California, CA, USA) statistics and data visualization packages. The SPSS was used to graphically represent and analyze the survival of the rats, as well as to carry out tests of group differences. MATLAB was used to calculate the values of exploratory variables and for data extrapolation (with a mixture of MathWorks developed, in-house and open-source code), as well as to visualize some data. PRISM was used for data visualization of all other data.

Results

A total of 12 animals out of 30 died after administration of the substances during the experiment. Three animals died shortly after the administration of ketamine-medetomidine, within 2 minutes after application, while the remainder died between 10 and 25 minutes after the administration. Although all the animals had received thiopental during previous procedures, only a third of the animals ($n = 4$) received thiopental before their death on a particular day; the remainder of the deceased animals ($n = 8$) died after receiving the ketamine-medetomidine mixture, and before the administration of thiopental.

No mortality was observed during the first two anaesthetic procedures (initial placement and reattachment of the coil spring and the first measurement). The first death was observed on day eight, when the affected animal required a reattachment of the coil spring. Mortality was exclusively observed in animals that required anaesthetic procedures more than once a week. Most of the animals (ten) which died before the end of the experiment in the first group required frequent anaesthetic procedures, because reattachments of the coil spring were required. In the second

group (in which the procedure was performed once weekly) only two animals died. Excessive salivation was observed in all the affected animals during anaesthesia. Additionally, respiratory distress was noticed in the animals after the administration of thiopental. The observation that the animals frequently urinated during anaesthesia, possibly indicating a release, and opening of sphincters, is also of note.

Hereinafter, the results are divided into three sections. First, an analysis of survival was carried out to verify that the survival of the two groups did in fact differ to a statistically significant degree. Second, three exploratory variables and their associations with survival were examined. Third, an extrapolation of the data was carried out in order to estimate the safe parameters for repeated ketamine, medetomidine and thiopental anaesthesia.

Analysis of Survival

When testing whether the two groups differed in the number of reattachments (with the inclusion of regular adjustments of the orthodontic appliance), the results of a Mann-Whitney test revealed that the two groups of rats differed significantly by the number of reattachments required ($U_{Mann-Whitney} = 39.5$, $W_{Wilcoxon} = 159.5$, Z = -3.169, $p = 0.002$). The median number of reattachments (and subsequently anaesthesias) needed was 8 (mean of 7.2) for the rats with removable appliances, and 5 (mean of 4.53) for the ones with fixed appliances. Non-parametric tests were used for all group comparisons, as the variables were found to be non-normally distributed.

The analysis of survival showed that a Cox model of survival using the group as a predictor was valid (better than null; $\chi^2 = 7.841$, $df = 1$, $p = 0.005$). The mean survival time for the rats with fixed orthodontic appliances, as estimated by the Kaplan-Meier method of survival analysis, was 38.73 days, while a mean of 32.40 days of survival was estimated for the rats with removable orthodontic appliances. More informatively, the medians of survival time for the two groups were 42 and 38 days, respectively. The raw data of rat survival by group is available in **Supplementary Table I** and **Supplementary File 1**.

The tests of equality of survival distributions (Table I, via the Kaplan-Meier method) showed that the survival functions differed for the two groups of rats (Figure 1, Panel A). The results of the analysis of survival using Cox's regression similarly showed that the hazard was significantly
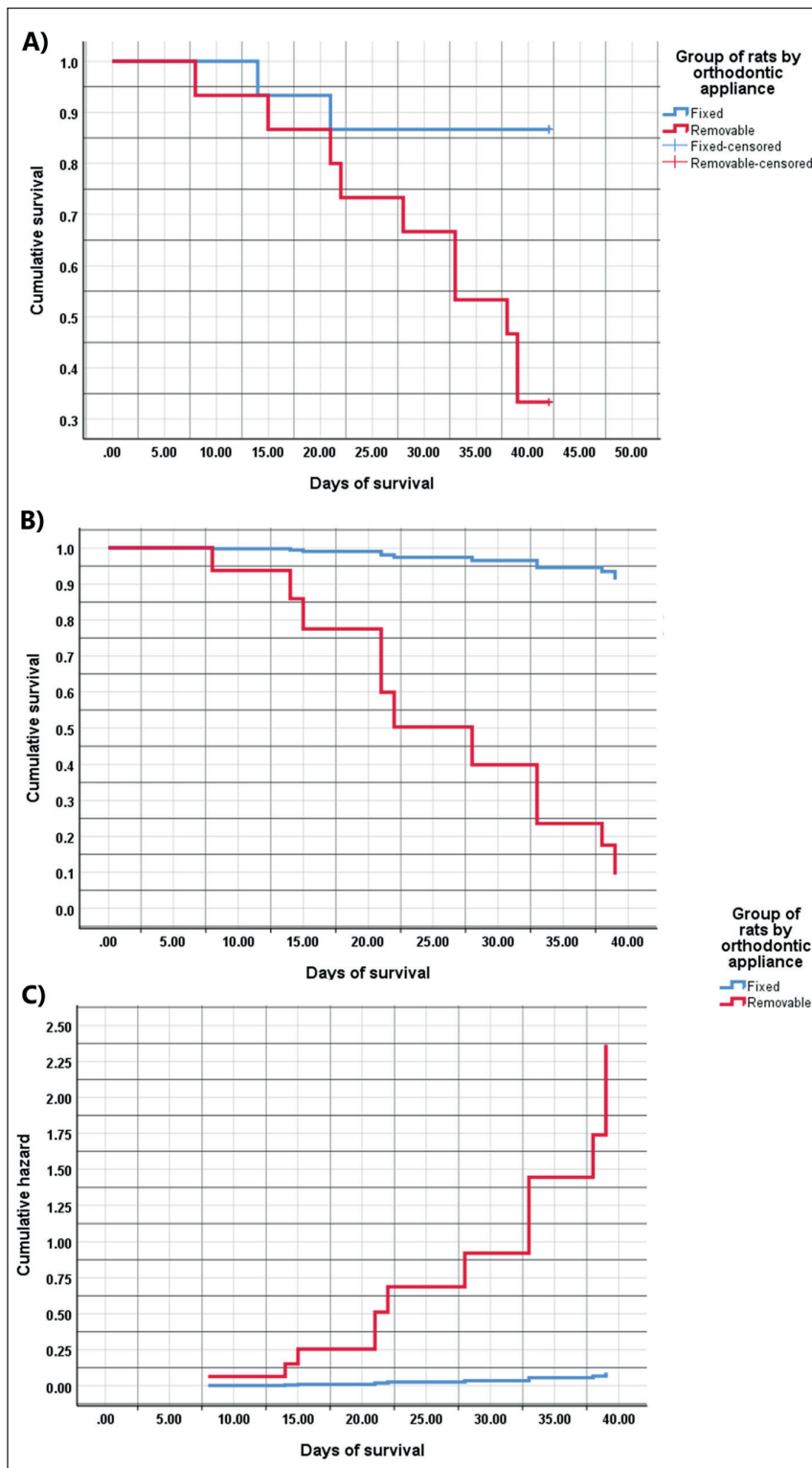
Figure 1. Cumulative survival and hazard curves for the two groups of male Wistar rats after receiving anaesthesia. A) shows the survival curve according to the Kaplan-Meier method. B) depicts the survival curve estimated with the Cox model and C) shows the cumulative hazard curves for the two groups of male Wistar rats, estimated by the Cox model. In all panels, the blue colour represents the fixed orthodontic appliance group and the red one represents the removable orthodontic appliance group.
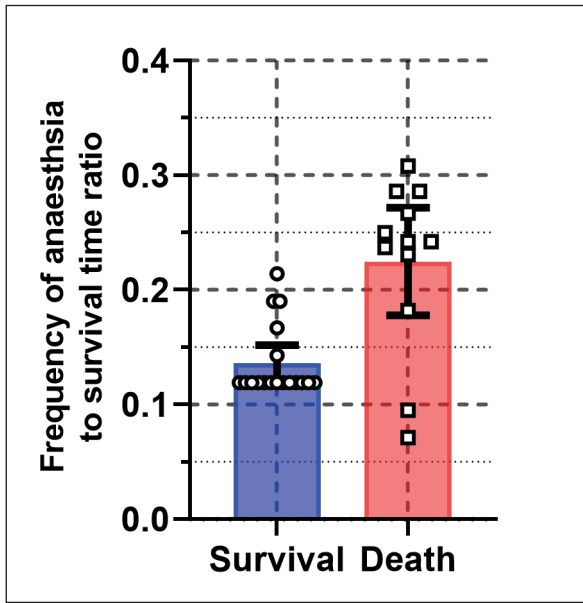
Figure 2. The plot of mean anaesthesia to survival time ratio for the rats that survived and the rats that died. The error bars represent 95% CI of means, while the points represent data from individual rats.

less for the rats with fixed orthodontic appliances ($e^B = 0.157$, $p = 0.017$, 95% CI $_{for\ eB} = 0.034 - 0.720$).

Exploratory Analyses

As a clear difference was observable in the survival of the two groups of rats, which can only be attributed to the increased frequency of the required reattachments and subsequent anaesthesia, several exploratory analyses were carried out to better explain the observed phenomenon. We attempted to explain and further explore the differences between the rats that died before the end of the experiment and those that survived to the end. The raw data and the descriptive statistics for all the variables are available in **Supplementary File 2** and **Supplementary Tables II, III, IV.**

Variable 1: Ratio of the frequency of anaesthesia to survival time:

The first possible explanatory factor is the ratio of the frequency of anaesthesia to survival time.

As this variable encompasses both the survival time of the remainder of the rats and the contrast with anaesthesia received during this time, it avoids being confounded by the correlation of survival time and the number of anaesthesias received (rats that live longer are logically exposed to more procedures due to the study protocol, $r_{Pearson} = 0.46$, $p = 0.011$). The mean ratio for the rats that survived was 0.136 anaesthesias per day ($SEM = 0.007$), and 0.225 for the rats that died ($SEM = 0.021$). This separation into survival and death during anaesthesia groups includes rats from both groups, with removable and fixed orthodontic appliances.

When testing for the differences in this parameter, the results of a Mann-Whitney test indeed showed that the rats that died before the end of the study had a significantly greater anaesthesia to survival time ratio than those that lived to the end of the study ($U_{Mann-Whitney} = 39$, $W_{Wilcoxon} = 210$, $Z = -3.048$, $p = 0.002$). The difference between the two is depicted in Figure 2.

From this ratio, a cut-off point above which all rats exposed to an amount of anaesthesia in a given time die, can be determined. The proposed cut-off point, based on the data from the present study, is illustrated in Figure 3.

The proposed cut-off point is 0.23, meaning that 0.23 anaesthesias per day is the point at which all rats will die in an experiment of similar length to ours (42 days) or longer. More intuitively, this could be interpreted such that should a rat receive an anaesthesia consisting of ketamine, medetomidine and thiopental every 4.3 days or more in an experiment, it will not survive to the end of the experiment. The possible use of this measure and others is discussed later in the paper.

Variable 2: The interval between the last two anaesthesia prior to death during anaesthesia (or termination at the end of the study):

The rats that survived had a mean interval between the last two anaesthesias of 5.44 days ($SEM = 0.25$), and those that died had an interval of 2.33 days ($SEM = 0.68$). When testing for the differences between the survivors and those that died

Table I. Results of tests of equality of survival distributions for the Kaplan-Meier method of survival analysis.

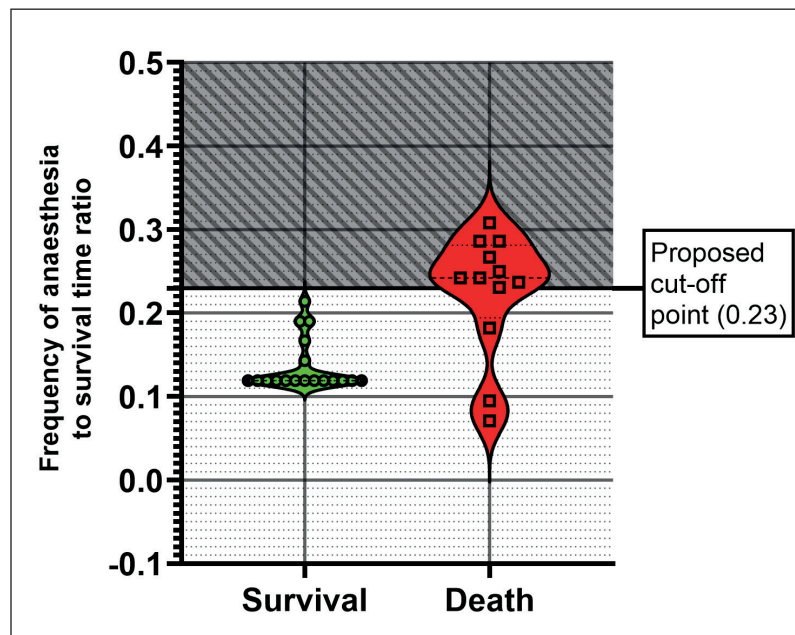|	χ^2	df	P
Log Rank (Mantel-Cox)	7.601	1	0.006
Breslow (Generalized Wilcoxon)	6.344	1	0.012
Tarone-Ware	6.980	1	0.008

Figure 3. Violin plots of survival rate of the male Wistar rats with both fixed and removable orthodontic appliances. Y-axis shows the ratio of frequency of anaesthesias to days of survival. X-axis shows the rats grouped by survival to the end of the study. In red, all the rats that died before the end of study are shown. Green shows the rats that survived. The proposed cut-off point is marked with a black line and the text box.

during the course of the study in this variable, a Mann-Whitney test again showed significant differences ($U_{Mann-Whitney}$ = 30.5, $W_{Wilcoxon}$ = 108.5, Z = -3.517, p = 0.0004). The difference in the mean interval is shown in Figure 4.

Variable 3: Mean interval between consecutive anaesthesia procedures:

The mean interval between consecutive anaesthesias of the surviving rats was 5.5 days (*SEM* = 0.22), while the deceased rats had an interval of 3.01 days (*SEM* = 0.43). Once more, significant differences between the surviving and the deceased rats could be observed (Mann-Whitney test; $U_{Mann-Whitney}$ = 24, $W_{Wilcoxon}$ = 102, Z = -3.804, p = 0.0001). The differences in the means of the time interval between anaesthesias can be seen in Figure 5.

Extrapolations of Safe Points According to the Ratio of Frequency of Anaesthesia and Survival Time

In this section we attempt to extrapolate the safe points in order to determine the values of each of the three exploratory variables where rats would be very unlikely to die due to anaesthesia. A simple linear extrapolation was used to proj-

ect the safe points, with the use of the MATLAB interp1 function. Next, to further the analysis, intersections of the extrapolation functions were calculated (using the MATLAB intersections function[16]) to determine the points where the lower and upper CIs of the variables were equal. The results of extrapolations are shown in Table II and **Supplementary Figures 1**, **2**, **3**.

Discussion

An uncommon and serious adverse reaction to the combined use of ketamine, an alpha 2 agonist and a barbiturate is reported in the present study, characterized by a high percentage of fatal outcomes. Although the main objective of the study was not the evaluation of the safety of this combination of anaesthetic drugs, but the evaluation of two different methods and protocols for orthodontic tooth movement, the results showed unexpected adverse reactions associated with frequently repeated anaesthesia.

In the present study, intraperitoneal injectable general anaesthesia containing a combination of agents was utilized. Although it may be considered as having a safety profile inferior to inha-
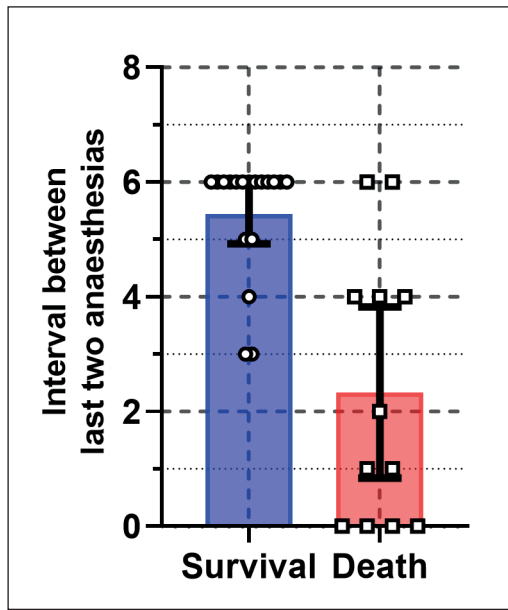
Figure 4. Plot of mean interval between the last two anaesthesia a rat received in days (Y-axis), grouped by outcome. The error bars represent 95% CI of means, while the points represent data from individual rats.

In the present study, all animals received thiopental as a second step during procedures after the ketamine-medetomidine application, but only four of them received thiopental in addition to ketamine-medetomidine before their death on a particular day. Eight animals died even before the administration of thiopental. This makes thiopental-related cardiorespiratory depression unlikely as the sole cause of mortality.

The second explanation could be attributed to the stress and modulated sensitivity of the rats' physiological systems by the frequent anaesthesia. As the frequent procedures needed to readjust the removable orthodontic appliance likely caused significant stress in the group of rats with removable appliances, the increased excitability of the rats due to stress-related changes could have made the rats more susceptible to adverse reactions. Moreover, a recent study[17] discovered

lation anaesthesia, the use of the latter was not feasible, as manipulation was required in the oral cavity of the experimental animals in order to gather data, or to mount or adjust the orthodontic appliance. The doses of ketamine and medetomidine used in our study (50 mg/kg b.w. and 0.067 mg/kg b.w., respectively) were lower than those reported and considered safe by Hedenqvist et al[14] (60 mg/kg b.w. and 0.4 mg/kg b.w., respectively).

As the combination of anaesthetic drugs used in the present study affects various biological systems, several different physiological reactions to them could explain the increased mortality observed in our study.

The first of these is that the incidence of cardiorespiratory depression induced by the combination of ketamine and medetomidine increases if barbiturates are administered concurrently[9,10].
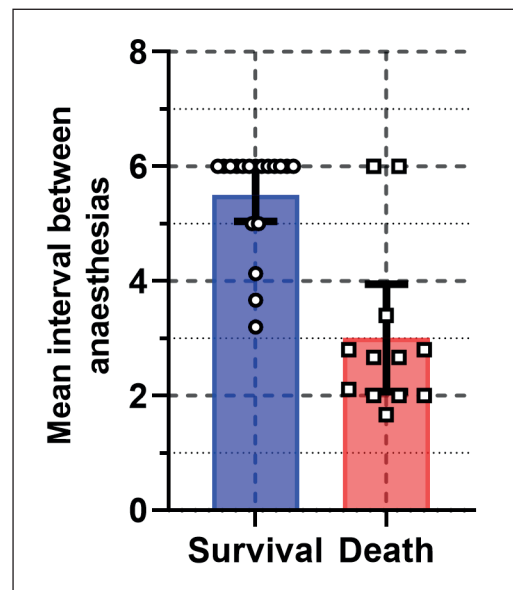

Figure 5. Plot of mean average interval between anaesthesia a rat received in days (Y-axis), grouped by outcome. The error bars represent 95% CI of means, while the points represent data from individual rats.

Table II. Linearly extrapolated means and 95%CIs for the exploratory variables.

	Mean	Lower 95% CI	Higher 95% CI	Intersection Y-value
Anaesthesia to survival ratio	0.047	0.064	0.032	0.0930
Interval between last two anaesthesias	8.55	9.02	8.09	7.0793
Mean interval between anaesthesias	7.99	8.01	7.97	7.8862

* Please note that because of the slopes of the lines connecting the 95% CIs of real data, the extrapolated high and low 95% CIs are inverted (the lower CI has higher values that the higher CI and vice versa).

long-term anxiogenic effects of ketamine in Wistar rats. Rats treated with 80 mg/kg of ketamine, without the addition of any other substance, exhibited increased anxiety-related behaviours in a variety of anxiety-related tasks two and seven days after the administration of ketamine. The authors proposed that the culprits of these anxiogenic effects of ketamine doses over 40 mg/kg were an increase in superoxidase dismutase, neurotoxicity to amygdalic neurons, and an increase in serotonergic transmission of the amygdala[17]. The levels of stress experienced by the rats in our study could thus have been further pronounced by these effects of ketamine, especially when combined with other substances that exert the liver.

Transient phenomena, denoting short-term changes in physiological parameters due to sudden changes in homeostasis, are the third of these possible explanations for increased mortality. In the anaesthetized animals, several transient phenomena were detected, among them increased heart rate, increased breathing and decreased breath volume. These transient changes may have played a role in the mortality of the rats, because if the rats were sensitized to the effects of anaesthetics, especially ketamine, an effect previously observed in Sprague Dawley rats by Rocha et al[18], a compounded transient effect may have increased the likelihood of cardiac and/or respiratory distress in the animals.

A transient increase in blood pressure, similar to animal studies, has also been reported in humans when ketamine was applied; the effect was attributed to the selective activation of the cardiovascular system which resulted in an increase in cardiac output. The blood pressure of subjects exposed to ketamine was found to exceed normal values in 20-35% of studies that reported adverse reactions to ketamine use in the treatment of depression[19]. The increase in both systolic and diastolic blood pressure was dose-dependent in humans and peaked at around 30-50 minutes after ketamine application. Blood pressure rose from 10% to 50% above pre-dose values and lasted 1-2 hours[20]. Albrecht et al[21] saw similar transient increases in mean arterial pressure (23%) in Wistar rats anaesthetized by the use of ketamine-xylazine in their study.

Such sudden changes could represent a shock to the already taxed cardiovascular system of the rats, which could have been compounded by the stress experienced due to the experimental protocols and thus resulted in increased mortality[22,23].

Although a slow application of anaesthesia cannot be applied in an animal experiment, when rapid onset of anaesthesia and subsequent rapid recovery is required, the gradual application of ketamine, as shown by Szarmach et al[24], could solve the problem of its transient adverse effects on the cardiovascular system. A ketamine-infused saline solution, administered over a 40-minute period, successfully evaded such transient effects in human subjects[24].

Increased salivation is another possible adverse reaction to ketamine anaesthesia and has been described in some animals in a previous study with rats[25]. It is possible that the large amounts of saliva observed in the affected rats obstructed the airway and caused subsequent suffocation, although the mortality of Wistar rats in ketamine anaesthesia due to excessive salivation is very rare. Thiopental and medetomidine may have served as contributing factors that potentiated combined cardiovascular tachycardial stimulation and respiratory depression and prevented the effective clearing of the rats' airways. It may also be possible for previous anaesthetic procedures to have induced delayed hypersensitive reactions to anaesthetic agents in the affected animals. This effect may be prevented by anticholinergics, although their routine use is questionable due to potential interference with studied drugs and/or procedures. In some strains of rat, salivation has been provoked by a combination of ketamine and hyperthermia, but not by the ketamine itself[12,25]. However, no such data was found for Wistar rats. In a study by Wixson et al[11], a combination of ketamine and the alpha-2 adrenergic agonist xylazine caused a dose-dependent depression in body temperature. When added to the ketamine-xylazine anaesthesia, any dose of pentobarbital resulted in profound hypothermia in the animals[11].

Repeated injections or prolonged exposure to ketamine is reported to cause a loss of the GABA-ergic phenotype of the parvalbumin interneurons[26]. Similarly, repeated exposure to ketamine in rats suppressed inhibitory synaptic transmission in the prefrontal cortex, producing biochemical changes in the GABAergic system that led to functional disinhibition[27]. This disinhibition due to repeated ketamine administration could have further compounded the effects of stress, resulting in hyper-excitable rats that were very vulnerable to cardiac and respiratory perturbations.

The general recommendations for future research, based on the data obtained in the current study, are that when anaesthesia is repeated frequently, the choice of anaesthetic agents should be selected to allow biotransformation and elim-

ination from the animal's body before the subsequent procedure is performed. The first measure implemented should be the use of anaesthetics at their lower dose range sufficient to induce and maintain anaesthesia for the desired time frame.

For the combination of ketamine and an alpha-2 agonist (medetomidine in our study), we recommend that the frequency of anaesthesia to time ratio, the time elapsed since the last anaesthesia and the mean time between consecutive anaesthesias, be taken into account when designing a study. All three of these exploratory variables were highly correlated (**Supplementary Table V**), which is not surprising as all deal with similar concepts, i.e., the time required between subsequent anaesthesias for the risks to the rat associated with the procedures to be minimized.

We propose that a ratio of anaesthesia to days of experiment (or survival days, when expected) that exceeds 0.23 (or approximately one anaesthesia per 4.3 days) will result in the rats dying, in an experiment of similar length to ours (42 days in this case) or longer (Figure 3). Furthermore, based on the extrapolation of our data (Table II), we propose three safe point values for repeated anaesthesia with ketamine and medetomidine. The first, based on the anaesthesia to survival ratio, is one anaesthesia per 21 days (21.27 raw value) when maximal survival of rats is required. The second, which provides a middle value between safety and practicality, is one anaesthesia per 11 days (10.745 raw value) and is the mean of the lower 95% CIs of the three extrapolated exploratory variables. The third, which yields the experimentally most practical value, is one anaesthesia per 8.5 days (8.573 raw value), which is the mean of the intersections of the 95% CIs interpolation functions.

In summary, several physiological effects may be responsible for increased mortality in more frequently anaesthetized rats. These are:
- The amplification of ketamine and medetomidine cardiovascular modulation by thiopental;
- Transient adverse cardiovascular reactions (increase in blood pressure, heart rate and breathing frequency, with a decrease in breath volume and modulated frequency);
- The accentuation of stress responses by the anxiogenic effects of ketamine;
- Increased salivation, which in combination with the respiratory depression of ketamine and medetomidine resulted in an obstruction of the airway and subsequent suffocation;

- A combination of stress due to frequent procedures being carried out and the transient effects, which would push the already stressed physiology of the rat over the tolerance limit;
- The loss of parvalbumin interneurons and subsequent disinhibition could play a role in the hyperexcitability of the rat's biological systems, possibly compounding the effects of the physiological stress.

Conclusions

Repeated anaesthesia containing ketamine, medetomidine and thiopental resulted in profoundly increased mortality of Wistar rats, with the less frequently anaesthetized group (median 5 times in 42 days) losing two members in the 42-day period and the more frequently anaesthetized group (median 8 times in 42 days) losing 10. Additionally, the parameters of the ratio of anaesthesia to days of survival and the interval length between consecutive anaesthesia were shown to be associated with mortality. Finally, a minimum safety period of 8.57 days between repeated anaesthesia is proposed.

Conflicts of Interest
All authors declare they have no financial nor personal relationships with people or organizations that could inappropriately influence this manuscript.

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