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Antagonistic effect of flumazenil on tiletamine-zolazepam-induced anaesthesia in Beagle dogs

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ABSTRACT: Benzodiazepines exert hypnotic/sedative effects through their inhibitory actions on the γ-aminobutyric acid receptor type A. Since flumazenil antagonises these effects through competitive inhibition of the receptor, it has been used to reverse the effect of benzodiazepines. The goal of this study was to characterise the antagonistic effect of flumazenil on anaesthesia induced by tiletamine-zolazepam in dogs. Nine healthy Beagle dogs (four males, five females) were used in this study. The dogs were administered 20 mg/kg of tiletamine-zolazepam intravenously and were then intravenously treated with saline solution (2 ml; control) or flumazenil twenty minutes after tiletamine-zolazepam administration at doses of 0.02, 0.04, 0.06, 0.08 or 0.16 mg/kg. Recovery times after the anaesthesia and cardiorespiratory variation were recorded for each dog. The results of this study indicate that the duration of reversal produced by doses of 0.04 and 0.06 mg/kg flumazenil was more effective than that produced by any of the other doses. In addition, sedation was rapidly reversible at 0.04 and 0.06 mg/kg without resedation. However, at doses of 0.08 and 0.16 mg/kg adverse effects such as shivering, rigidity and opisthotonos were observed. Thus, treatment with flumazenil at doses of 0.04 and 0.06 mg/kg could successfully reverse the anaesthetic effects induced by tiletamine-zolazepam.

Keywords: antagonization; flumazenil; tiletamine-zolazepam; cardiorespiratory effect; $GABA_A$

Tiletamine-zolazepam (TZ) is an anaesthetic combination composed of the two drugs in a 1 : 1 ratio that is usually indicated for anaesthesia for diagnostic and surgical procedures in veterinary medicine. After intravenous TZ administration, lateral recumbency and tracheal intubation can be achieved within one minute in dogs (Gomez-Villamandos et al. 2013). However, TZ is not recommended in patients with respiratory or cardiovascular diseases, and supplementary oxygen must be administered to prevent hypoxaemia. Tiletamine is a long-acting, dissociative anaesthetic agent and has a greater analgesic effect than ketamine (Lin 2007). Zolazepam, a benzodiazepine agonist, enhances the effect of tiletamine on the central nervous system and decreases skeletal muscle hyperactivity. In high doses, TZ depresses the respiratory system and recovery may be complicated; therefore, proper attention to anaesthesia is required in dogs with respiratory and neurological disease (Lin et al. 2009).

Flumazenil (F) has been used to antagonise the side effects of benzodiazepines, which include sedation, impaired recall, psychomotor impairment and ventilatory depression. On the other hand, the adverse effects of flumazenil itself include seizures and resedation, and even death has been reported (Penninga et al. 2016). Therefore, careful administration of flumazenil is recommended for patients receiving continuous high-dose benzodiazepine therapy (Moore et al. 2014). A previous study has shown that flumazenil alone could successfully and

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safely reverse the anaesthetic effect induced by TZ in pigs, but pigs are less sensitive to dissociatives, and therefore antagonisation of TZ by flumazenil does not reveal as many side effects as in dogs (Lee and Kim 2012). To the best of our knowledge, there is no study that has investigated the use of flumazenil to reverse tiletamine-zolazepam anaesthesia in dogs. Our hypothesis was that flumazenil would reverse tiletamine-zolazepam-induced sedation. Thus, the aims of the present study were to evaluate the antagonistic effect of flumazenil on anaesthesia induced by tiletamine-zolazepam in dogs.

MATERIAL AND METHODS

Experimental animals and study design. Nine healthy Beagle dogs (four males, five females) weighing 9 ± 1.2 kg (mean ± SD) and aged 1.5 ± 0.5 years were used in this study. The dogs were fasted for six hours before anaesthesia and rested for two weeks after the experiment. For each of the nine dogs, six different treatments were administered in a randomised order at the rate of one treatment every 14 days. The study was approved by the Institutional Animal Care and Use Committee (IACUC, CBNUR-469-12), and performed in the Laboratory Animal Research Center of Chungbuk National University, Republic of Korea. An intravenous catheter was inserted into the cephalic vein and the dogs were administered tiletamine-zolazepam (Zoletil 50, Virbac Laboratories, Carros, France) intravenously at a high dose of 20 mg/kg of body weight. Twenty minutes after the tiletamine-zolazepam administration, the dogs received saline solution (TZ group) or flumazenil (Flunil, Bukwang Pharmaceutical Co., Ltd., Seoul, Republic of Korea) (TZF group) intravenously. The TZF group was subdivided into TZF1, TZF2, TZF3, TZF4 and TZF5 groups depending on the flumazenil dose (0.02 mg/kg, 0.04 mg/kg, 0.06 mg/kg, 0.08 mg/kg and 0.16 mg/kg, respectively). During anaesthesia, the dogs were positioned in left lateral recumbency. Prior to each anaesthetic treatment, sterile 22-gauge catheters (BD IV Catheter, Becton Dickinson, Republic of Korea) were inserted into the dorsal pedal artery for arterial blood pressure measurement and blood sampling. All dogs received oxygen via a regular face mask and oxygen saturation (sO_2) was measured.

Cardiorespiratory parameters . Heart rate (HR), systolic arterial pressure (SAP), mean arterial pressure (MAP) and diastolic arterial pressure (DAP), respiratory frequency (*f*R), rectal temperature (T) and pulse oximetry (SpO_2) were recorded with a patient monitor (Cardell 9600 hd, Midmark, Dayton, USA) before anaesthesia and after intravenous injection of tiletamine-zolazepam at time points of 5, 10, 20, 30 and 40 min. Heart rate (HR) and respiratory frequency (*f*R) were measured using lead ECG and capnography.

Using a three-syringe technique from the three-way stopcock connected directly to the arterial catheter, blood samples were collected for blood gas analysis. Approximately 1 ml of blood sample was discarded from the first syringe, after which 0.5 ml were collected in heparinised (25 IU) 1-ml syringes anaerobically. The catheter was then flushed to ensure patency. Blood gas analysis was performed using a self-calibrating analyser within two minutes of sample collection. Values were normalised to body temperature, which was recorded before each measurement. Partial arterial pressure of carbon dioxide ($PaCO_2$), arterial pH, partial arterial pressure of oxygen (PaO_2) and oxygen saturation (sO_2) were measured with a blood analyser (i-STAT, Heska Corp., Pleasant Hill, USA) at T0 (5 min after administration of tiletamine-zolazepam) and T1 (20 min after injection of flumazenil or saline solution).

Recovery. To examine the effect of flumazenil on recovery in a dose-dependent manner, the times required for the appearance of the following behaviours were recorded: head up, sternal recumbency, standing and walking. The head up time was defined as the time interval from the moment of injection of TZ to the first moment when the animal attempted to lift its head a few centimetres above the ground. The sternal recumbency time was defined as the time interval between the injection of TZ and when the animal became completely recumbent. The standing time was defined as the time interval between the injection of TZ and when the animal stood without assistance for more than 10 seconds. The walking time was defined as the time interval between the injection of TZ and when the animal could walk without assistance. The overall quality of anaesthetic recovery was scored according to four distinct criteria of recovery as described in Table 1.

Statistical analysis. The results are presented as means ± standard deviation. The variation between groups was analysed using the Kruskal-Wallis test

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Table 1. Recovery scoring system in dogs

Score 0	Poor/unsuccessful: seizures and opisthotonus with extended limbs
Score 1	Fair: salivation, shivering, howling
Score 2	Good: anxious but responsive to external stimuli
Score 4	Excellent: calm and responsive to external stimuli

and the Mann-Whitney *U*-test. Differences within groups were examined with one-way analysis of variance (ANOVA) followed by Duncan's post-hoc tests. All statistical tests were performed using a computer statistical package (Statistical Package for the Social Sciences, version 19.0; SPSS Inc., Chicago, USA).

RESULTS

In all groups, the cardiorespiratory parameters showed a significant decrease below baseline after the administration of TZ (Tables 2–4). In the TZ-treated group, HR decreased within 5 min after the administration and the value remained consistently below the baseline for 40 min. The TZF-treated groups showed decreased HR for 20 min upon administration, but the HR increased above the baseline after flumazenil was injected. There were significant differences in HR between the two groups with the exception of the 0.02 mg/kg TZF group at 30 and 40 min after TZ administration (Table 2). Blood pressures were significantly increased in all groups after tiletamine-zolazepam administration when compared with the baseline values. Following tiletamine-zolazepam administration, the mean values obtained for SAP, MAP and DAP were significantly higher at 10 to 40 minutes when compared with baseline values ($P < 0.05$). There was no significant difference in blood pressure between the TZ and TZF groups. Rectal temperature (T) was significantly decreased after administration of TZ in all groups. Respiratory frequency (*f*R) also decreased after the administration of TZ in all groups. In the TZ group, *f*R decreased within 5 min after TZ administration and remained consistently under the baseline for 40 min. In the TZF groups, *f*R was decreased for 20 min but rose above the baseline after flumazenil administration. The levels of SpO_2 in the TZF groups were significantly higher than in the TZ group at 30 and 40 min, respectively (Table 2). The levels of $PaCO_2$ in the TZ groups were significantly higher than that in the TZF groups (Table 3). PaO_2 and sO_2 were increased after the administration of flumazenil in all of the TZF groups, and the levels were significantly higher than those of the TZ group (Table 3). In addition, at high doses of flumazenil, the TZF groups (0.08 mg/kg, 0.16 mg/kg flumazenil) showed a significantly lower score that the other groups (Figure 1).

The recovery period was significantly shorter in the groups receiving 0.04 mg/kg–0.16 mg/kg flumazenil than in the TZ group. However, the recovery time of the low-dose TZF group (0.02 mg/kg flumazenil) was not significantly different from that of the TZ group (Table 4). In addition, at high doses of

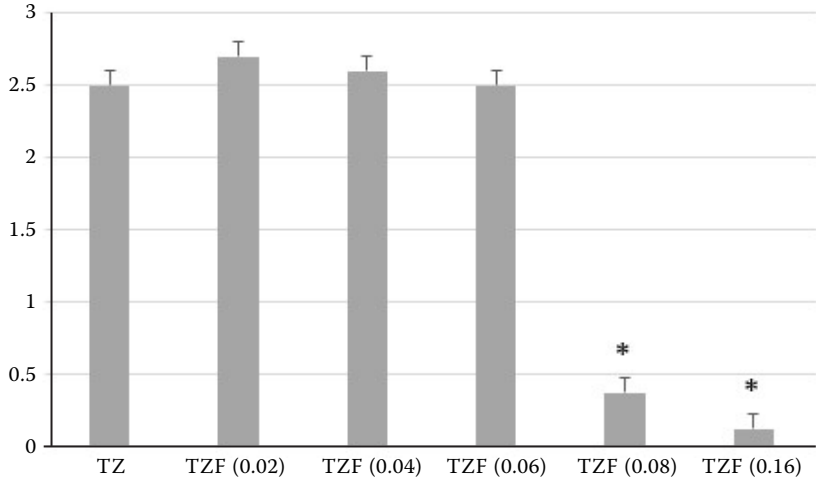

Figure 1. Recovery score in Beagle dogs after administration of tiletamine-zolazepam (TZ) or tiletamine-zolazepam-flumazenil (TZF)

*$P < 0.05$

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Table 2. Heart rate (HR), blood pressure (systolic (SAP), mean (MAP) and diastolic (DAP) arterial pressure), rectal temperature (T), respiratory rate (*f*R) and SpO_2 in dogs after the administration of tiletamine-zolazepam (TZ) or tiletamine-zolazepam-flumazenil (TZF); data are expressed as the mean ± SD (n = 6)

	Group	Pre	5 min	10 min	20 min	30 min	40 min
HR (beats/minute)	TZ	116 ± 8	112 ± 6	110 ± 7*	100 ± 5*	103 ± 5*	106 ± 4*
	TZF (0.02)	119 ± 6	110 ± 8	109 ± 9*	101 ± 7*	105 ± 8*	106 ± 8*
	TZF (0.04)	115 ± 6	112 ± 8	108 ± 9*	100 ± 7*	119 ± 8†	116 ± 8†
	TZF (0.06)	115 ± 7	112 ± 9	105 ± 9*	99 ± 8*	125 ± 5†	129 ± 7†
	TZF (0.08)	112 ± 10	110 ± 8	102 ± 6*	101 ± 8*	148 ± 9*‡	150 ± 9*‡
	TZF (0.16)	114 ± 10	106 ± 8	102 ± 6*	103 ± 10*	165 ± 5*‡	188 ± 7*‡
SAP (mm Hg)	TZ	112 ± 17	122 ± 11	120 ± 13	131 ± 16*	132 ± 14*	132 ± 18*
	TZF (0.02)	122 ± 16	122 ± 12	120 ± 13	141 ± 13*	142 ± 9*	142 ± 15*
	TZF (0.04)	116 ± 14	122 ± 15	130 ± 13	142 ± 11*	142 ± 10*	132 ± 19*
	TZF (0.06)	116 ± 17	123 ± 14	130 ± 14	146 ± 12*	142 ± 11*	142 ± 16*
	TZF (0.08)	122 ± 11	122 ± 9	130 ± 20	151 ± 15*	146 ± 14*	142 ± 11*
	TZF (0.16)	124 ± 12	132 ± 16	145 ± 27	155 ± 18*	148 ± 16*	153 ± 21*
MAP (mm Hg)	TZ	93 ± 14	98 ± 11	101 ± 12	112 ± 11*	111 ± 13*	112 ± 14
	TZF (0.02)	93 ± 11	98 ± 13	101 ± 9	101 ± 12*	120 ± 14*	112 ± 14
	TZF (0.04)	95 ± 12	98 ± 11	99 ± 12	111 ± 14*	110 ± 17*	102 ± 16
	TZF (0.06)	95 ± 12	103 ± 10	110 ± 19	126 ± 16*	122 ± 11*	121 ± 10*
	TZF (0.08)	96 ± 15	102 ± 10	120 ± 19	136 ± 15*	126 ± 11*	112 ± 10
	TZF (0.16)	97 ± 11	112 ± 14	125 ± 14	126 ± 16*	128 ± 14*	122 ± 15
DAP (mm Hg)	TZ	73 ± 12	78 ± 15	81 ± 12	92 ± 14*	91 ± 16*	92 ± 15*
	TZF (0.02)	78 ± 16	78 ± 13	81 ± 19	81 ± 18	80 ± 19	92 ± 14*
	TZF (0.04)	75 ± 11	78 ± 16	79 ± 12	91 ± 17*	90 ± 16*	92 ± 12*
	TZF (0.06)	75 ± 12	93 ± 13	90 ± 14	102 ± 12*	102 ± 11*	101 ± 10*
	TZF (0.08)	76 ± 15	82 ± 10	90 ± 19	106 ± 15*	106 ± 11*	92 ± 10*
	TZF (0.16)	75 ± 14	93 ± 11	90 ± 17	106 ± 18*	102 ± 19*	101 ± 20*
T (°C)	TZ	39.0 ± 0.4	38.9 ± 0.4	38.6 ± 0.5	38.6 ± 0.4	38.4 ± 0.3	38.4 ± 0.3*
	TZF (0.02)	38.5 ± 0.2	38.4 ± 0.2	38.3 ± 0.2	38.2 ± 0.4	38.2 ± 0.1	38.1 ± 0.2*
	TZF (0.04)	38.7 ± 0.2	38.8 ± 0.2	38.5 ± 0.3	38.4 ± 0.3	38.3 ± 0.2	38.2 ± 0.2*
	TZF (0.06)	38.8 ± 0.2	38.7 ± 0.2	38.5 ± 0.4	38.4 ± 0.3	38.3 ± 0.2	38.2 ± 0.2*
	TZF (0.08)	38.7 ± 0.5	38.4 ± 0.5	38.1 ± 0.5	38.4 ± 0.3	38.3 ± 0.2	38.1 ± 0.3*
	TZF (0.16)	38.8 ± 0.5	38.6 ± 0.5	38.5 ± 0.4	38.4 ± 0.5	38.1 ± 0.2	38.1 ± 0.3*
*f*R (beats/minute)	TZ	23 ± 2	13 ± 2*	9 ± 1*	11 ± 2*	11 ± 2*	16 ± 3
	TZF (0.02)	24 ± 2	14 ± 3*	13 ± 3*	14 ± 3*	12 ± 2*	15 ± 3
	TZF (0.04)	26 ± 3	13 ± 3*	15 ± 2*	16 ± 3*	31 ± 5	32 ± 4
	TZF (0.06)	23 ± 4	11 ± 2*	13 ± 2*	14 ± 2*	28 ± 6	29 ± 6
	TZF (0.08)	24 ± 3	16 ± 2*	16 ± 2*	15 ± 3*	26 ± 5	33 ± 4
	TZF (0.16)	23 ± 2	13 ± 2*	11 ± 3*	13 ± 2*	26 ± 5	32 ± 4
SpO_2 (%)	TZ	98.5 ± 2.1	94.0 ± 3.9*	94.5 ± 4.2*	94.5 ± 1.7*	94.0 ± 1.4*	94.3 ± 0.5*
	TZF (0.02)	98.5 ± 2.6	94.3 ± 3.1*	94.3 ± 4.3*	94.3 ± 1.8*	97.5 ± 0.8	97.5 ± 1.3
	TZF (0.04)	98.5 ± 2.1	94.0 ± 3.0*	94.0 ± 3.8*	96.8 ± 2.5	97.3 ± 1.4†	98.3 ± 0.5†
	TZF (0.06)	98.5 ± 2.1	95.5 ± 2.3*	95.0 ± 2.8*	96.8 ± 1.5	97.8 ± 2.4†	98.5 ± 0.8†
	TZF (0.08)	98.6 ± 3.1	95.3 ± 4.3*	94.3 ± 3.4*	98.0 ± 0.5†	98.8 ± 1.1†	98.3 ± 0.5†
	TZF (0.16)	98.46 ± 2.1	93.5 ± 3.3*	95.3 ± 4.1*	98.5 ± 1.2†	97.8 ± 2.1†	98.0 ± 0.8†

TZ = tiletamine-zolazepam, TZF = tiletamine-zolazepam-flumazenil

*Significantly different ($P < 0.05$) from the baseline

†Significantly different ($P < 0.05$) from TZ

‡Significantly different ($P < 0.03$) from TZ

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Table 3. Changes in arterial pH (pH_a), partial arterial pressure of CO_2 ($PaCO_2$), partial arterial pressure of O_2 (PaO_2) and oxygen saturation (sO_2) after flumazenil injection in tiletamine-zolazepam-anaesthetised Beagle dogs; data are expressed as the mean ± SD (n = 9)

Group	pH_a		$PaCO_2$		PaO_2		sO_2	
	T0	T1	T0	T1	T0	T1	T0	T1
TZ	7.36 ± 0.01	7.30 ± 0.01*	43.5 ± 0.62	57.4 ± 0.85*	64.3 ± 1.71	72.0 ± 2.16*	88.3 ± 0.96	85.5 ± 3.70
TZF (0.02)	7.36 ± 0.01	7.29 ± 0.04*	42.5 ± 0.80	59.9 ± 1.00*	57.5 ± 1.73	98.3 ± 2.50*†	77.0 ± 1.83	97.0 ± 2.16*
TZF (0.04)	7.35 ± 0.02	7.33 ± 0.01	42.0 ± 2.11	45.9 ± 1.76†	48.0 ± 1.41	92.3 ± 2.63*†	80.8 ± 1.89	96.5 ± 1.73*
TZF (0.06)	7.37 ± 0.01	7.36 ± 0.03	40.6 ± 1.94	38.6 ± 1.76†	50.3 ± 1.50	96.3 ± 2.63*†	78.5 ± 2.38	96.5 ± 1.29*
TZF (0.08)	7.35 ± 0.01	7.37 ± 0.04	43.9 ± 2.47	41.3 ± 2.73†	48.8 ± 1.79	100.0 ± 2.64*†	84.8 ± 2.50	98.0 ± 0.82*
TZF (0.16)	7.36 ± 0.01	7.37 ± 0.02	40.2 ± 1.92	37.9 ± 1.85†	50.3 ± 1.89	98.0 ± 2.16*†	86.0 ± 3.11	98.3 ± 1.26*

T0 = 5 min after administration of tiletamine-zolazepam, T1 = 20 min after injection of flumazenil or saline solution, TZ = tiletamine-zolazepam, TZF = tiletamine-zolazepam-flumazenil

*Significantly different (P < 0.05) from the baseline

†Significantly different (P < 0.05) from TZ

flumazenil, the TZF groups (0.08 mg/kg, 0.16 mg/kg flumazenil) showed adverse effects including salivation, shivering, seizures, howling, hacking coughs, and opisthotonus with the limbs extended.

DISCUSSION

Flumazenil injection at doses of 0.04 and 0.06 mg/kg quickly and effectively reversed tiletamine-zolazepam-induced sedation.

Complete recovery from anaesthesia with tiletamine-zolazepam was prolonged (100.3 ± 7.4 min) and was considered smooth when used without an antagonist. However, flumazenil administration at doses of 0.04, 0.06, 0.08 and 0.16 mg/kg resulted in a significantly reduced recovery period. Flumazenil antagonises the action of benzodiazepines on the central nervous system by acting as a competitive inhibitor of the benzodiazepine recognition site on GABA (γ-aminobutyric acid receptor) (Moore et al. 2014). When administered immediately after surgery, flumazenil shortens the time required for recovery from the sedative effects of surgical anaesthetics. It also reverses the effects of overdoses of benzodiazepines including tiletamine-zolazepam. Flumazenil has been used to antagonise sedation, impaired recall, psychomotor impairment and ventilatory depression resulting from benzodiazepine overdoses (Moore et al. 2014). In the current study, recovery from sedation in dogs administered flumazenil at doses of 0.04 and 0.06 mg/kg was smooth with minimal adverse reactions and with no reversion to sedation, whereas dogs administered doses of 0.08 and 0.16 mg/kg experienced multiple adverse effects such as salivation, shivering, seizures, howling, hacking coughs and opisthotonus with the limbs extended. These pronounced side effects were probably caused by the adverse effects of a benzodiazepine antagonist on the central nervous system and the continued effects of tiletamine (Nakamura et al. 2004).

Recent studies have reported similar results in humans, in whom high doses have been found to cause alertness and panting in some cases (Nejad et al. 2017). Some investigators have suggested that flumazenil may increase intracranial pressure and should therefore be avoided in patients with space-occupying intracranial lesions (Mordel et al. 1992). However, flumazenil at doses of 0.04 and 0.06 mg/kg was sufficiently effective in the present study.

Table 4. Recovery times (in minutes) in Beagle dogs after administration of tiletamine-zolazepam (TZ) or tiletamine-zolazepam-flumazenil (TZF); data are expressed as the mean ± SD (n = 9)

Group	Head up	Sternal recumbency	Standing	Walking
TZ	65.8 ± 7.4	69.8 ± 4.03	93.5 ± 6.03	100.3 ± 7.41
TZF (0.02)	70.8 ± 4.2	74.5 ± 4.20	99.8 ± 6.85	110.5 ± 8.35
TZF (0.04)	38.0 ± 3.1†	43.0 ± 2.94†	59.0 ± 4.97†	82.5 ± 4.20†
TZF (0.06)	34.0 ± 4.6†	43.5 ± 5.45†	58.0 ± 5.94†	81.0 ± 6.98†
TZF (0.08)	35.3 ± 2.7†	41.3 ± 3.86†	70.3 ± 4.35†	82.5 ± 6.19†
TZF (0.16)	35.3 ± 3.8†	48.5 ± 3.87†	64.3 ± 4.92†	72.5 ± 5.92†

TZ = tiletamine-zolazepam, TZF = tiletamine-zolazepam-flumazenil

†Significantly different (P < 0.05) from TZ

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The mean standing time and mean total recovery time in the groups that received these doses were significantly shorter than those in the control group. We also determined the effects of flumazenil on cardiorespiratory parameters and recovery in dogs sedated by tiletamine-zolazepam. Thirty minutes after administration of tiletamine-zolazepam, HR significantly decreased to 91–96% of baseline values. Although mean HR in the control group continued to fall gradually to the lowest value of 100 beats/min, these changes were described to remain in the normal range in dogs and cats (Campbell and Chapman 2000).

Flumazenil reversed these tiletamine-zolazepam-induced changes in HR within 10 min after injection, and its effect on HR was dose-dependent. The lowest dose of flumazenil (0.02 mg/kg) did not return the HR to baseline levels, while doses of flumazenil of 0.04 and 0.06 mg/kg reverted HR to approximately baseline levels. The tachycardia was probably caused by the central stimulant effect of flumazenil, the continued effect of tiletamine and by an effect on the peripheral vascular bed, which resulted in transient vasodilatory hypotension and a reflex increase in HR (Soleimanpour et al. 2010).

The most significant findings of this study with respect to respiration and gas exchange were the decreases in *f*R, SpO_2, PaO_2 and arterial pH and the increases in $PaCO_2$ following the administration of tiletamine-zolazepam. These parameters in the TZ group changed with time, and were significantly lower (*f*R, SpO_2, PaO_2, arterial pH) or higher ($PaCO_2$) than the baseline values at 30 and 40 min following induction of anaesthesia. In contrast, *f*R, SpO_2 and PaO_2 did not show significant changes from the baseline values from 5 to 40 min in the TZF groups. In addition, *f*R, SpO_2 and PaO_2 values in the TZF groups were significantly higher at 30 and 40 min than those in the TZ group. Thus, flumazenil antagonised the cardiorespiratory effects induced by tiletamine-zolazepam in dogs.

In conclusion, the intravenous injection of flumazenil effectively reversed the sedation induced by tiletamine-zolazepam (20 mg/kg, intravenous) in Beagle dogs. Flumazenil injection at doses of 0.04 and 0.06 mg/kg quickly and effectively reversed tiletamine-zolazepam-induced sedation. Optimal results were observed at doses of 0.04 and 0.06 mg/kg. Recovery from sedation was quick and smooth, and only minimal adverse effects were seen with either dose.

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