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Antagonistic Effects of Flumazenil on Tiletamine-Zolazepam Induced Anesthesia in Dogs

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Abstract : The purpose of this study was to determine the antagonistic effects of flumazenil on anesthesia induced with tiletamine/zolazepam in dogs. The anesthetic effects (sedation, analgesic, muscle relaxation, posture and auditory response score), vital signs (heart rate, respiratory rate and rectal temperature) and blood biochemistry (glucose (GLU), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST)) were examined as indicators of the antagonistic effects. A total of 6 clinically healthy mongrel dogs were used in this study. The dogs in TZ group received administration of tiletamine/zolazepam 10 mg/kg IV. The dogs in TZF group received administration dose of TZ 10 mg/kg IV followed by the administration of flumazenil 0.1 mg/kg 20 minutes after administering a TZ 10 mg/kg dose. There were significant differences in the recovery of anesthesia between the groups. The GLU level in the TZF group after the administration of flumazenil was significantly higher than that of the TZ group. There was a larger change in the HR in the TZF group than in the TZ group until 30 minutes after flumazenil administration. The sternal recumbency, standing and walking times of the TZF group were faster than those of the TZ group. In conclusion, flumazenil showed antagonistic effect against tiletamine/zolazepam in dogs. When recovering from anesthesia, flumazenil reduced sternal recumbency, standing and walking times.

Key words **:** antagonisim, dog, flumazenil, tiletamine/zolazepam.

Introduction

In veterinary medicine, although tiletamine/zolazepam (TZ) has the disadvantage of storage and anesthesia (9), its utilization has increased owing to its safety compared with other anesthetic drugs.

Zolazepam complements tiletamine to produce abroad range of immobilization and anesthetic induction in wild animals and domestic animals (8) that is superior to ketamine (9). Active research in Korea (7,11) and overseas (3,13) have demonstrated the superiority of TZ. Many practitioners acknowledge it as a familiar anesthetic drug. The anesthetic utilization of TZ has increased but many practitioners use doxapram or an oxygen supply for anesthetic recovery (6). Therefore, there is insufficient knowledge about flumazenil, which is a good efficient benzodiazepine antagonist.

The characteristics and recovery process of flumazenil are similar to those of benzodiazepine. Flumazenil is an imidazobenzodiazepine derivative that binds to the GABA receptor complex and blocks it (12). Benzodiazepine is safe when the appropriate dosage is used (6) but prolonged sedation and respiratory depression have been reported. In this emergency situation, flumazenil can reduce the risks associated with benzodiazepine (10).

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Toxicity studies in animals at Hoffman-LaRoche laboratories using acute and chronic administration have reported that flumazenil is well tolerated by all routes of administration, with signs of toxicity reported only at very high doses (1).

It was reported that an over dosage of diazepam (2.0 mg/kg IV) or of midazolam (1.0 mg/kg IV) can antagonize with flumazenil (0.08 mg/mg IV) (12). However, there are no reports of the antagonistic effects of flumazenil against TZ in dogs. The purpose of this study was to determine the antagonistic effects of flumazenil on anesthesia induced in dogs with tiletamine/zolazepam.

Materials and Methods

Experimental animals

A total of 6 clinically healthy mongrel dogs (5.2 ± 2.3 years old, 5.2 ± 2.2 kg BW, Female 3, Male 3) were used in this study. The experimental dogs were fed commercial dog food (ANF, ANF specialties, USA) for 2 weeks before the experiment. The dogs were fasted for 24 hours before administering the drugs in order to prevent any possible adverse effects associated with anesthesia. All the anesthetic drugs were injected into the cephalic vein. The dogs were assigned to the control group (tiletamine/zolazepam; TZ group: 6 dogs) and treatment group (tiletamine/zolazepam/flumazenil; TZF group: 6 dogs). There was a crossover experimental design in these dogs. The dogs were investigated after administration. In addi-

tion, the dogs were rested for 2 weeks. TZ was administered again followed 20 minutes later by flumazenil. The dogs were then examined. This study was performed in accordance to the rules of the Ethics Committee for Experimental Animals, Chungnam National University.

Anesthesia

All the anesthetic drugs were injected into the cephalic vein through a 24G × 3/4" IV catheter (BD IV catheter, Becton Dickinson Korea, Korea).

The dogs in TZ group received administration dose of tiletamine/zolazepam 10 mg/kg IV (Zoletil®, Virbac Laboratories, France). The dogs in TZF group received administration dose of TZ 10 mg/kg IV followed by the administration of flumazenil 0.1 mg/kg (Flunil®, Bu Kwang Pham, Korea) 20 minutes after administering a TZ 10 mg/kg dose.

Scores of anesthetic effects

The score of the anesthetic effects (sedation, analgesia, muscle relaxation, posture and auditory response score) was determined using response scores of 0, 1, 2 or 3 (Table 1).

Complete immobilization was defined as a lack of response to handling. The score of the anesthetic effects was evaluated subjectively before anesthesia and 1, 5, 20, 30, 60 and 90 minutes during anesthesia. A score was given to each category. The data is reported as the mean ± SD.

Blood biochemistry

Blood samples (1 ml) were collected at each time point by venepuncture from the jugular vein, and measured before anesthesia (Pre) and 1, 5, 20, 30, 60 and 90 minutes after anesthesia. The samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU) and the total proteins (TP) using an autoanalyer (Vetest 8008 Blood Chemistry Analyzer; IDEXX)

Vital signs

The heart rate was measured using an electrocardiogram (Pulscan-Component, Scionic Co., LTD., USA). The dogs' respiratory rate was measured by observing their abdominal movements. The dogs' rectal temperature was measured with a digital thermometer.

The heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded before anesthesia and 1, 5, 20, 30, 60 and 90 minutes after anesthesia.

Table 1. Criteria and score of the anesthetic effects

Criteria	Score	Observation
Sedation score		
	0	Normal
	1	Mild sedation (head down, strong palpebral reflex, normal eye position)
	2	Moderate sedation (moderate palpebral reflex, partial ventromedial eye rotation)
	3	profound sedation (palpebral reflex, absence, complete ventromedial eye rotation)
Analgesia score (by pinching of the skin in the inguinal area)		
	0	Normal (productive flight response)
	1	Mild (exaggerated movements of limbs and trying to get up)
	2	Moderate (slight movements of the limbs and trying to get up)
	3	Profound (lack of response)
Muscle relaxation score		
	0	Normal jaw and leg tone
	1	Mild relaxation of jaw and leg tone
	2	Moderate relaxation and leg tone
	3	Profound relaxation of jaw and leg tone
Posture score		
	0	Standing
	1	Sitting or ataxic
	2	Sternal recumbency
	3	Lateral recumbency
Auditory response score (response to noise created by a handclap close to the animals ears)		
	0	Normal response
	1	Mild decrease in response (eye movement with body movement)
	2	Moderate decrease in response (eye movement without body movement)
	3	Profound decrease in response (no movement)

Induction and recovery

The dogs in both groups became rapidly anesthetized after an IV injection of TZ and they all became laterally recumbent within 15 seconds without any signs of excitement.

The recovery times (head up, sternal recumbency, standing and walking time) were measured using a digital timer from complete immobilization to when normal behavior was observed. The head up time is defined as the time interval between the injection of TZ and the first attempt made by the animal to lift its head a few centimeters above the ground. The sternal recumbency time was defined as the time interval between the injection of TZ and when the animal become 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.

Statistical analysis

All statistical analysis between the TZ and TZF groups were carried out using Microsoft Excel® (Microsoft, USA) and SAS 9.1. All the data is expressed as the mean ± SD. All the data was analyzed using a student' t-test. A p value < 0.05 was considered significant.

Results

Scores of anesthetic effects

The sedation score of the TZ group increased significantly from the baseline to 2.2 ± 0.4, 1.2 ± 0.8 and 0.7 ± 0.5 at 1, 20 and 30 minutes, respectively ($P < 0.05$). In contrast, the score of the TZF group increased significantly from the baseline to 2.5 ± 0.5, 2.5 ± 0.5, 1.8 ± 0.4 and 1.3 ± 0.5 at 1, 5, 20 and 30 minutes, respectively ($P < 0.05$). The degree of the sedation score at 5 and 30 minutes was significantly higher in the TZF than the TZ group ($P < 0.05$) (Table 2).

The analgesic score of the TZ group increased significantly from the baseline to 2.8 ± 0.4, 2.8 ± 0.4, 2.7 ± 0.5, 2.7 ± 0.5 and 1.8 ± 1.0 at 1, 5, 20, 30 and 60 minutes, respectively ($P < 0.05$). On the other hand, the score of the TZF group increased significantly from the baseline to 2.5 ± 0.5 and 2.2 ± 0.9 at 20 and 30 minutes, respectively ($P < 0.05$).

The muscle relaxation score of the TZ group increased significantly from the baseline to 1.8 ± 0.4 and 1.0 ± 0.9 at 5 and 20 minutes, respectively ($P < 0.05$). On the other hand, the score of the TZF group increased significantly from the baseline to 2.3 ± 0.5, 2.5 ± 0.5, 2.3 ± 0.5 and 1.3 ± 0.8 at 1, 5, 20 and 30 minutes, respectively ($P < 0.05$). The degree of muscle relaxation at 5, 20 and 30 minutes was significantly higher in the TZF group than in the TZ group ($P < 0.05$).

The posture score of the TZ group at 60 minutes increased significantly from the baseline to 1.7 ± 1.0 ($P < 0.05$). In contrast, the score of the TZF group increased significantly from the baseline to 2.7 ± 0.8 at 30 minutes ($P < 0.05$).

The auditory response score of the TZ group increased significantly from the baseline to 2.8 ± 0.4, 2.7 ± 0.8, 2.3 ± 0.8 and 1.8 ± 1.2 at 5, 20, 30 and 60 minutes, respectively ($P < 0.05$). However, the score of the TZF group increased significantly from the baseline to 2.8 ± 0.4, 2.8 ± 0.4 and 2.2 ± 1.0 at 1, 20 and 30 minutes, respectively ($P < 0.05$).

Serum Chemical Profiles (GLU/ TP/ ALT/ AST)

The GLU level in the TZF group after the administration of flumazenil, at 20, 30 and 90 minutes was significantly higher than that of the TZ group ($P < 0.05$) (Table 3). The TP level in the TZ group decreased significantly from the baseline at 5, 20 and 30 minutes. On the other hand, the TP level in the TZF group decreased significantly from baseline at 5 minutes ($P < 0.05$). The ALT level was similar in the TZ and TZF group ($P < 0.05$). The AST level in the TZ group increased

Table 2. Scores for the anesthetic effects (sedation. analgesia, muscle relaxation, posture, and auditory response) after the administration of TZ or TZF

Effect	Group	Pre	1min	5min	20min	30min	60min	90min
Sedation	TZ	0.0 ± 0.0	2.2 ± 0.4*	2.0 ± 0.0	1.2 ± 0.8*	0.7 ± 0.5*	0.2 ± 0.4	0.0 ± 0.0
	TZF	0.0 ± 0.0	2.5 ± 0.5*	2.5 ± 0.5*+	1.8 ± 0.4*	1.3 ± 0.5*+	0.2 ± 0.4	0.0 ± 0.0
Analgesia	TZ	0.0 ± 0.0	2.8 ± 0.4*	2.8 ± 0.4*	2.7 ± 0.5*	2.7 ± 0.5*	1.8 ± 1.0*	0.7 ± 1.2
	TZF	0.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.5*	2.2 ± 0.9*	1.3 ± 1.5	0.0 ± 0.0
Muscle relaxation	TZ	0.0 ± 0.0	2.0 ± 0.0	1.8 ± 0.4*	1.0 ± 0.9*	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0
	TZF	0.0 ± 0.0	2.3 ± 0.5*	2.5 ± 0.5*+	2.3 ± 0.5*+	1.3 ± 0.8*+	0.5 ± 0.8	0.0 ± 0.0
Posture	TZ	0.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	1.7 ± 1.0*	0.8 ± 1.0
	TZF	0.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	2.7 ± 0.8*	0.8 ± 1.0	0.0 ± 0.0
Auditory response	TZ	0.0 ± 0.0	3.0 ± 0.0	2.8 ± 0.4*	2.7 ± 0.8*	2.3 ± 0.8*	1.8 ± 1.2*	0.5 ± 0.8
	TZF	0.0 ± 0.0	2.8 ± 0.4*	3.0 ± 0.0	2.8 ± 0.4*	2.2 ± 1.0*	0.7 ± 1.2	0.0 ± 0.0

Data is expressed as the mean ± SD (n = 6).
*Significantly different ($P < 0.05$) from the baseline.
+Significantly different ($P < 0.05$) from the TZ group.
TZ: tiletamine/zolazepam; TZF: tiletamine/zolazepam /flumazenil

Table 3. The change in GLU, TP, ALT, AST levels in TZ and TZF groups

	Group	Pre	1min	5min	20min	30min	60min	90min
G L U	TZ	71.8 ± 11.7	76.7 ± 13.1	79.8 ± 10.8	68.5 ± 7.9	71.8 ± 16.8	75.7 ± 11.2	75.7 ± 17.8
	TZF	86.0 ± 22.2	86.3 ± 15.1	89.2 ± 15.1	86.0 ± 12.4^{+}	100.5 ± 15.7^{+}	87.5 ± 12.1	99.5 ± 13.5^{+}
T P	TZ	5.4 ± 0.4	5.0 ± 0.6	5.1 ± 0.4*	4.0 ± 1.3*	4.7 ± 0.9*	5.0 ± 1.1	4.98 ± 1.0
	TZF	5.4 ± 0.4	5.1 ± 0.6	5.2 ± 0.4*	4.8 ± 0.5	5.4 ± 0.6	5.0 ± 1.1	4.8 ± 1.1
A L T	TZ	45.0 ± 17.1	42.5 ± 9.7	43.5 ± 12.4	37.0 ± 8.2	41.0 ± 12.1	38.8 ± 8.7	42.3 ± 11.6
	TZF	39.0 ± 7.0	38.2 ± 7.8	41.7 ± 7.7	37.5 ± 5.8	40.7 ± 9.3	40.0 ± 10.7	41.2 ± 9.5
A S T	TZ	29.8 ± 9.7	37.0 ± 11.7*	35.2 ± 6.0	23.0 ± 12.8	21.7 ± 7.1	23.2 ± 13.4	35.0 ± 16.5
	TZF	28.2 ± 9.7	25.8 ± 11.2	27.0 ± 7.4	23.3 ± 8.6	29.5 ± 6.3	23.2 ± 10.6	23.3 ± 5.3

Data is expressed as the mean ± SD (n=6).
*Significantly different ($P < 0.05$) from the baseline.
$^{+}$Significantly different ($P < 0.05$) from the TZ group.
ALT: alanine aminotransferase; AST; aspartate aminotransferase; GLU: glucose; TP: total protein; TZ: tiletamine/zolazepam; TZF: tiletamine/zolazepam /flumazenil

Table 4. The change in the HR, RR, RT in the TZ and TZF groups

	Group	Pre	1min	5min	20min	30min	60min	90min
HR	TZ	118.5 ± 19.6	190.7 ± 37.4*	201.2 ± 15.3*	175.8 ± 46.9	164.3 ± 28.3*	163.8 ± 23.1*	141.3 ± 25.9
	TZF	97.5 ± 13.4	196.8 ± 23.6*	203.8 ± 24.9*	182.8 ± 17.3*	179.8 ± 24.4*	154.3 ± 17.8*	139.7 ± 33.8*
RR	TZ	21.8 ± 5.9	35.6 ± 10.6*	29.2 ± 11.7	31.2 ± 8.2	34.2 ± 14.4	28.8 ± 13.8	25.4 ± 10.5
	TZF	27.7 ± 11.8	51.3 ± 28.8	41.0 ± 30.6	45.3 ± 30.7	35.6 ± 15.0	36.0 ± 16.0	31.5 ± 7.8
RT	TZ	39.0 ± 0.2	38.6 ± 0.3*	38.1 ± 0.3*	37.5 ± 0.3*	37.2 ± 0.5*	37.0 ± 1.2*	37.3 ± 1.3*
	TZF	39.1 ± 0.4	39.0 ± 0.6	38.7 ± 0.5^{*+}	38.3 ± 0.4^{*+}	38.5 ± 0.5^{*+}	38.9 ± 0.9^{+}	39.1 ± 0.7^{+}

Data is expressed as the mean ± SD (n = 6).
*Significantly different ($P < 0.05$) from the baseline.
$^{+}$Significantly different ($P < 0.05$) from the TZ group.
HR: heart rate; RR: respiratory rate; RT: rectal temperature; TZ: tiletamine/zolazepam; TZF: tiletamine/zolazepam /flumazenil

significantly from the baseline at 1 minute, but it remained within the normal range ($P < 0.05$).

Changes in the heart rate, respiratory rate and rectal temperatures

There was a larger change in the HR in the TZF group than in the TZ group until 30 minutes after flumazenil administration. The HR decreased gradually in the TZ group. The heart rate in the TZ group increased significantly from the baseline to 190.7 ± 37.4, 201.2 ± 15.3, 164.3 ± 28.3 and 163.8 ± 23.1 at 1, 5, 30 and 60 minutes, respectively ($P < 0.05$). In TZF group, the HR increased significantly from the baseline to 196.8 ± 23.6, 203.8 ± 24.9, 182.8 ± 17.3, 179.8 ± 24.4, 154.3 ± 17.8 and 13.7 ± 33.8 at 1, 5, 20, 30, 60 and 90 minutes, respectively ($P < 0.05$) (Table 4).

The RR of the TZ group increased from the baseline to 1minute ($P < 0.05$). However, the respiratory rate of the TZF group at 1, 5 and 20 minutes was higher than that of the TZ group and it increased slightly from 30 to 90 minutes ($P < 0.05$).

The RT in the TZF group was more stable during the course of anesthesia than the TZ group. The RR in the TZ group decreased from the baseline to 38.6 ± 0.3, 38.1 ± 0.3, 37.5 ± 0.3, 37.2 ± 0.5, 37.0 ± 1.2 and 37.3 ± 1.3, at 1, 5, 20, 30, 60 and 90 minutes respectively ($P < 0.05$). However, the RR of the TZF group decreased from the baseline to 39.7 ± 0.5, 38.3 ± 0.4, 38.5 ± 0.5 at 5, 20 and 30 minutes, respectively ($P < 0.05$). There was significant difference between TZ and TZF group at the time of 5, 20, 30, 60 and 90 minutes ($P < 0.05$).

Induction and recovery

All dogs became rapidly anesthetized within 15 seconds. The head up time of the TZ group was 3 minutes faster than that of the TZF group. However, the sternal recumbency, standing and walking times of the TZF group were 6, 12 and 22 minutes faster than the TZ group, respectively (Table 5).

Discussion

Benzodiazepines are used for their sedative, anxiolytic, amnesic, anticonvulsant, and muscle relaxant properties (8). Although benzodiazepines are considered safe when used properly, prolonged sedation and respiratory depression have been reported (1,9,10,12).

Table 5. Recovery time from anesthesia in TZ and TZF groups

	TZ	TZF
Head up	29.7 ± 9.5	32.8 ± 14.4
Sternal recumbency	50.7 ± 19.9	44.7 ± 9.7
Standing	82.0 ± 23.6	66.2 ± 20.1
Walking	96.2 ± 28.0	74.5 ± 25.0

Data are expressed as mean ± SD (n = 6)
TZ: tiletamine/zolazepam; TZF: tiletamine/zolazepam /flumazenil

Flumazenil is an imidazobenzodiazepine antagonist that is structurally related to midazolam, and readily antagonizes the sedative, respiratory depressant, anxiolytic, muscle relaxant, anticonvulsant, amnestic and anesthetic effects of benzodiazepines (1,13). Flumazenil is not highly protein bound (< 40%) and has a relatively high hepatic extraction ratio (0.6). The drug undergoes hepatic metabolism and the primary metabolite is inactive (12). The initial signs of reversal are generally observed within 1 min, depending on the flumazenil dose and degree of benzodiazepine-induced sedation. Its duration of action after a single intravenous injection varies from 15 to 140 minutes depending on the dose (5). An elimination half-life of 0.4 to 1.3 hours has been reported for dogs (1,12). Flumazenil is much shorter than any of the benzodiazepines commonly used for sedation or anesthesia (1). With the half-life of midazolam and diazepam being approximately 2.4 hours and 10 times longer, there is the possibility of a recurrence of the effects of benzodiazepine, such as sedation or respiratory depression, after the flumazenil has been eliminated.

In order to manage resedation, flumazenil administration may be repeated using the same titration method every 20 min, provided the dose does not exceed 1 mg/20 min or 3 mg/hr (13). There is some concern that flumazenil might precipitate acute withdrawal syndrome in patients who have been taking benzodiazepines for a long time (5). Adverse reactions, including convulsions, can occur when flumazenil is used with ketamine or tiletamine (7). Hence, at least 20 minutes should elapse after the last dose of a dissociative anesthetic before flumazenil is administered (7).

In this study, the posture score of the TZ group decreased 60 minutes after administration, but the TZF group showed a decrease 30 minutes after the injection. In addition, the TZF group showed a lower auditory response score than the TZ group 30 minutes after administration.

Compared with the other benzodiazepines, zolazepam causes the least central nervous system (CNS) depression, and there is some concern regarding the recovery reaction of flumazenil against zolazepam (9).

Sedation occurred in the TZ group after 1, 20 and 30 minutes, and in TZF group after 1, 5, 20 and 30 minutes. The TZF group had a higher sedation score than the TZ group at 30 minutes. In addition, there was a significant muscle relaxation score at 5 and 20 minutes in the TZ group and 1, 5, 20 and 30 minutes in the TZF group. The TZF group showed a higher muscle relaxation score than the TZ group until 30 minutes after administration. Interestingly, the muscle relaxation increased and had the duration in the TZF group than in the TZ group. In this study, the level of sedation and muscle relaxation were similar to previous research in that imidazobenzod iazepines comprise a range of partial agonists and competitive antagonists. These have some but not all of the benzodiazepine-like actions, i.e., they antagonize one or more of the effects of benzodiazepine, combine agonist and antagonist properties. The range of activities exerted by this series of benzodiazepine ligands suggests that the various actions of benzodiazepine may be separable (2). Flumazenil may have limited agonist properties including mild anxiolytic and anticonvulsant effects. Although, flumazenil can act as a partial or inverse agonist, it rarely acts as an agonist not antagonist (2,5).

In the anesthetic stage, the glucose level in the TZF group increased significantly at 20, 30 and 90 minutes. It was previously reported that flumazenil does not affect the blood biochemistry (1). Moreover, the hyperglycemia is due to the recovery from anesthesia and is related to hyperthermia.

The total protein in the TZ group was lower at 5, 20 and 30 minutes, but the total protein in the TZF group was significantly lower at 5 minutes. Nevertheless, both groups were in the similar values of total protein. The ALT level of the TZ and TZF groups were in the normal range, and there were no significant differences between the two groups. The AST of the TZ group increased 1 minute after administration. Both groups were in the normal range. The blood chemistry of both the tiletamine/zolazepam group did not show any changes in the total protein, ALT and AST, except for glucose.

In a previous study, TZ 10 mg/kg were administered to dogs every day for 7 days. The hematology (PCV, WBC, RBC, TP, fibrinogen), serum chemistry (ALT, AST, BUN, creatinine) and histopathology test (liver, kidney) showed a mild increase in the WBC and fibrinogen, which is a common sign after an injection (11). It was reported that flumazenil does not affect the hematobiochemistry (14).

The heart rates of the TZ and TZF groups increased significantly from one to 5 minutes, then decreased at 20, 30, 60 and 90 minutes. However, it showed higher value during anesthesia than the baseline. This is similar to previous research results showing an increased heart rate in dogs given TZ with no premedication. In addition 2 mg/kg of tiletamine administered i.v. resulted in an increased heart rate in unanaesthetized dogs (3).

The heart rate increased during anesthesia with TZ (3) but the administration of flumazenil had little effect on the heart rate, which is similar to previous reports showing no significant effect on the cardiovascular system from the sedation to recovery stages (1,14).

The respiratory rates in the TZ and TZF group increased 1 minute after administration, then decreased at 5, 20, 30, 60 and 90 minutes. However, it showed higher respiratory rates than the baseline during anesthesia. There is limited information on the effects of zolazepam on the respiratory system in

animals. One study reported an increased respiration rate in most dogs after a tiletamine/zolazepam dose ranging from 6.6 to 19.8 mg/kg i.v (9). In the present study, flumazenil did not increase the respiratory rate from tiletamine. The respiratory rate showed similar trends to previous reports (14), but no depressed respiration like benzodiazepine (10,14).

The body temperature of the TZ group decreased at 1, 5, 20, 30 and 60 minutes, compared with the baseline. However, the TZF group had a normal body temperature in 5, 20, 30, 60 and 90 minutes. The hypothermia observed in the TZ group was similar to previous reports showing that hypothermia can occur after the administration of tiletamine/zolazepam. It is believed to occur in response to muscle relaxation (9). Flumazenil did not affect vasoconstriction (1). Moreover, the body temperature maintenance in the TZF group was a normal body reaction during the recovery from anesthesia.

The TZ group showed steady analgesic scores at 1, 5, 20 and 30 minutes but a decrease after 60 minutes. In contrast, the TZF group showed a similar decrease but had lower analgesic scores. There was some concern that the TZF group would suffer increased pain due to the antagonistic effects of flumazenil because benzodiazepine does not have analgesic effect, and tiletamine and zolazepam complemented each other.

In the recovery stage, the head up time of the TZF group (32.8 ± 14.4) was 3 minutes slower than the TZ group (29.7 ± 9.5). The sternal recumbency, standing and walking times of the TZF group was an average 6 (44.7 ± 9.7 vs. 50.7 ± 19.9), 16 (66.2 ± 20.1 vs. 74.5 ± 25.0) and 22 (82.0 ± 23.6 vs. 96.2 ± 28.0) minutes faster than the TZ group, respectively. Flumazenil showed quick recovery against benzodiazepine, which is similar to a previous study (4).

Anderson *et al* (1) reported that the administration of flumazenil produced normal arousal within 60 seconds of the IV injection for benzodiazepine sedation.

In this study, the anesthetic recovery time of the TZF group was shorter than the TZ group. However, it lasted for 1 hour after flumazenil administration, which is in contrast to the delayed recovery reported by Anderson *et al* (1). It is believed that Zoletil® is composed not only of zolazepam, which is benzodiazepine, but also tiletamine.

In this study, the administration of flumazenil 0.1 mg/kg to Zoletil® 10 mg/kg showed a partial antagonistic effect. And flumazenil added to incrase the sedative and muscle relaxation. However, in terms of the secondary effect, the body temperature and glucose level recovered from Zoletil® quickly.

In conclusion, flumazenil showed antagonistic effect against Zoletil®. Therefore, it reduced the anesthetic recovery time. Flumazenil acts as an efficient antagonist and reduces the delayed recovery, which is an adverse effect caused by the general health status or individual sensitivity differences in canine anesthetic practice.

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Tiletamine-Zolazepam에 의한 개의 마취에서 Flumazenil의 길항효과

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요 약 : 본 연구는 tiletamine/zolazepam 대조군과 대조군에 benzodiazepine의 길항제인 flumazenil을 투여한 실험군의 마취효과와 혈액생화학, Vital sign, 마취회복시간에 대하여 비교하였다. 건강한 6마리의 개들(평균 체중 5.2 ± 2.2)이 실험에 이용되었다. Tiletamine/zolazepam 을 투여하였고 20분 뒤에 benzodiazepine의 길항제인 flumazenil 0.1 mg을 투여하였다. 마취효과 (Sedation, analgesia, muscle relaxation, posture and auditory response score), Vital sign (심박수, 호흡수, 체온), 혈액생화학 (GLU, TP, ALT, AST) 검사들이 두 그룹에서 tiletamine/zolazepam 투여전, 투여후 1, 5, 20, 30, 60 그리고 90분에 실시되었다. 또한 마취회복시간 (head up, sternal recumbency, standing and walking times)은 부동화 상태에서 각 행동양식이 보이는 시간까지 측정되었다. Analgesia score는 투여 후 20분과 30분에, posture score는 투여 후 30분에 그리고 auditory response score는 투여 후 1분과 20분에 TZF그룹이 TZ그룹보다 유의하게 낮아 benzodiazepine에 대한 flumazenil의 길항효과를 나타냈다. 평균 심박수는 두 그룹 모두 투여 후 1분부터 급상승하여 유의하게 정상 심박수 보다 높았다. 평균 호흡수는 TZF그룹이 TZ그룹보다 유의하지 않았으나 높은 호흡수를 보였다. 결론적으로 개에서 flumazenil의 투여는 tiletamine/zolazepam에 대한 길항작용을 나타내었다. 마취로 부터의 회복에 있어서, flumazenil은 sternal recumbency, standing 및 walking times를 단축시켰다.

주요어 : 길항작용, 개, flumazenil, tiletamine/zolazepam