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Effects of Injectable Anesthetics on Fluorescein Retinal Angiographic Phases in Dogs

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Abstract : This study compared the effect of injectable combinations of anesthetics on each of the fluorescein angiographic phases in order to determine the most useful anesthetic combination for the procedure. Acepromazine-ketamine (AK), xylazine-ketamine (XK), diazepam-ketamine (DK) and zolazepam-tiletamine (ZT) group were administered randomly to 8 dogs with a two-week interval between different combination doses. The vital signs including the heart rate and arterial pressure were measured before anesthesia and every five minutes during anesthesia. Serial angiographic images were obtained after injecting a sodium fluorescein dye (25 mg/kg) and the onset time of arterial phase (AP), arteriovenous phase (AVP), early venous phase (EVP) and late venous phases (LVP) were recorded. The onset time of the AP, AVP and EVP were significantly slower in the AK and XK groups than in the DK and ZT groups. The total duration of the AP and AVP in the AK group was significantly longer than those in the ZT group. The heart rates were significantly higher in the DK and ZT groups. The arterial pressure was significantly higher in the AK and XK groups ($p<0.05$). There were significant differences in each angiographic onset time and duration depending on the changes in the heart rates and arterial pressure. The AK and XK groups showed a long angiographic duration allowing an accurate evaluation. Overall, it is believed that AK and XK are more useful for performing fluorescein retinal angiography than DK and ZT.

Key words **:** fluorescein retinal angiography, acepromazine, ketamine, xylazine, diazepam, zolazepam-tiletamine, dog.

Introduction

Fluorescein is widely used dye for the diagnosing vascular defects. Fluorescein angiography has been used both clinically and experimentally to characterize abnormal vascular conditions of the human and animal fundus (16, 18).

The control of the patient with the eye in the axial position for a proper angiogram is essential during investigation (1). Fluorescein angiography in veterinary ophthalmology is mandatory under deep sedation or even general anesthesia. This may have an effect on the fluorescein angiographic duration through cardiovascular stimulation (13).

Various anesthetics have been used for canine fluorescein retinal angiography including acepromazine - ketamine, xylazine (4), diazepam - ketamine, propionylpromazine – propofol (16), halothane, isoflurane and sevoflurane (13). However, there are little informations on the individual effects of each anesthetic agent.

This study compared the effects of acepromazine - ketamine, diazepam - ketamine, xylazine - ketamine and zolazepam - tiletamine combinations on each fluorescein angiographic phase in order to determine the most useful anesthetic combination for fluorescein retinal angiography in dogs.

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Materials and Methods

Animals

Eight healthy dogs of both genders, with an average body weight of 4.3 ± 0.6 kg (mean ± SD), were used in this study. All dogs were screened and considered healthy based on a complete physical examinations prior to this study. A commercial pellet diet (Purina pellet®, Purina Co., Korea) and water were supplied *ad libitum*, but food was withheld for 12 hours before each study. The experiments adhered to the strict guidelines of the "Guide for the Care and Use of Laboratory Animals" of Seoul National University (Seoul, South Korea).

Anesthesia and monitoring

All dogs were treated with each of the 4 anesthetic protocols. The 4 different anesthetic protocols were used randomly in each dog with an interval of two weeks between each procedure. An intravenous catheter was positioned into the right cephalic vein for an injection of sodium fluorescein dye and fluid.

Acepromazine - ketamine (AK) group : Pre-anesthetic, atropine 0.04 mg/kg (Atropine sulfate®, Huons Co, Korea) was administered subcutaneously. Ten minutes later, anesthesia was induced with acepromazine 0.2 mg/kg (Sedaject®, Samwoo chemical, Korea) and ketamine 10 mg/kg (Yuhan Ketamine 50®, Yuhan Co, Korea) intravenously according to Plumb (5).

Xylazine - ketamine (XK) group : The anesthetic protocol was similar to the AK group, with the exception that xylazine 1.1 mg/kg (Rompun®, Bayer Korea, Korea) and ketamine 10 mg/kg were administered intravenously (12).

Diazepam - ketamine (DK) group : Premedication was performed in a similar manner to the AK group. Diazepam 0.5 mg/kg (Merode®, Donghwa Co, Korea) and ketamine 10 mg/kg were administered intravenously according to the method reported by Lin (12).

Zolazepam - tiletamine (ZT) group : Premedication was similar to the AK group. Zolazepam 2.5 mg/kg and tiletamine 2.5 mg/kg (Zoletil 50®, Virbac, Korea) was administered intravenously (3).

The following variables were recorded before anesthesia; heart rate (HR, beat/min), respiratory rate (RR, breaths/min), systolic arterial pressure (SP, mmHg), diastolic arterial pressure (DP, mmHg), mean arterial pressure (MAP, mmHg), and rectal temperature (RT, °C). During maintenance, these variables were recorded every five minutes after anesthesia using an anesthesia-monitoring device (Datex-Ohemeda S/5™, Datex-Ohemeda Division, Instrumentarium Co, Finland).

Clinical evaluation of anesthetics

A clinical evaluation was performed during anesthesia based on the following clinical signs that might affect the interruption factors for the angiographic procedure : eyeball dropping, third eyelid protrusion, nystagmus, head movement and tongue movement.

Fluorescein angiography

The pupils were dilated with 1 % tropicamide (Oculotropic®, Samil Pharm, Korea) 20 minutes before administering the anesthetics. Fundus fluorescein angiography was performed using a fundus camera (Kowa Hand-Held Fundus Camera GENESIS™, Kowa Co, Tokyo, Japan) at 10 minutes after administering the injectable anesthetics. The dog was placed in a sling restraint device. The eyelid was kept opened with an eye speculum. The cornea was kept moist with an artificial tear (Lacure®, Samil Pharm, Korea). Sodium fluorescein dye (Fluorescite® 10 %, Alcon, USA) was administered intravenously at a dose of 25 mg/kg with 40 ml/min (4). Serial retinal angiographic images were taken with a fundus camera every 1 second for the first 20 seconds after injecting the fluorescein dye with a color slide film (Elite chrome, 100 ASA, Kodak Ltd, Korea).

The following fluorescein angiographic phases were examined; arterial phase (AP : when the dye appeared in the retinal arteries), arteriovenous phase (AVP : when the dye appeared in the retinal capillaries), early venous phase (EVP : when the laminar flow of the dye appeared in the veins), and late venous phase (LVP : when the veins were totally filled with the dye). D_1, D_2 and D_3 were defined as AVP - AP, EVP - AVP and LVP – EVP, respectively.

Statistical analysis

All statistical analysis were carried out using SPSS® (SPSS for Windows Release 11.5 Standard Version, SPSS Ins., USA) ; $p<0.05$ was considered significant. The difference in the HR, RR, MAP, SP and DP within the groups was analyzed using a paired t-test. The mean differences in the HR, RR, MAP, SP and DP between the groups were analyzed using one-way ANOVA and Tukey HSD. One-way ANOVA was used to compare the angiographic variables (AP, AVP, EVP, LVP, D_1, D_2 and D_3) between the groups. A linear regression test was used to confirm the relationship between each phase and vital signs. A linear by linear association test was used to evaluate the angiographic interruption factors during anesthesia.

Results

Fluorescein angiography

After injecting the 10 % sodium fluorescein dye, the AP, AVP, EVP and LVP are as follows : 7.20 ± 1.13, 10.60 ± 1.47, 14.43 ± 2.16 and 19.14 ± 2.94 in the AK group, 7.40 ± 0.65, 10.40 ± 1.13, 13.78 ± 1.25 and 18.03 ± 1.38 in the XK group, 5.65 ± 0.77, 8.48 ± 1.20, 11.40 ± 1.52 and 15.73 ± 1.63 in the DK group, and 5.35 ± 0.75, 7.63 ± 0.66, 9.80 ± 1.20 and 13.73 ± 0.91 in the ZT group, respectively (mean ± SD seconds). The D_1, D_2 and D_3 were : 3.41 ± 0.72, 3.81 ± 1.03 and 4.71 ± 1.08 in the AK group, 3.01 ± 0.59, 3.36 ± 1.05 and 4.25 ± 0.91 in the XK group, 2.83 ± 0.69, 2.93 ± 0.85 and 4.33 ± 0.70 in the DK group, 2.28 ± 0.39, 2.18 ± 0.61 and 3.93 ± 0.66 in the ZT group, respectively (mean ± SD seconds).

Fig 1 shows a representative angiograph of each phase. Tables 1 and 2 show the onset time and duration of each phase. All angiographic phases showed significantly slower progress in the AK and XK groups than in the DK and ZT groups. The onset time of the AP, AVP and EVP was significantly slower in the AK and XK groups than in the DK and ZT groups. The LVP of the AK group was significantly slower than that of the ZT group. Each angiographic duration showed a similar tendency with the angiographic phases. D_1 and D_2 of the AK group were significantly different from those of the ZT group ($p<0.05$).

Cardiopulmonary variables

The heart rates in the DK and ZT groups increased significantly after administering the anesthetics (Fig 2). There were no significant changes in the respiratory rates in all groups (Fig 3). The arterial blood pressure decreased significantly

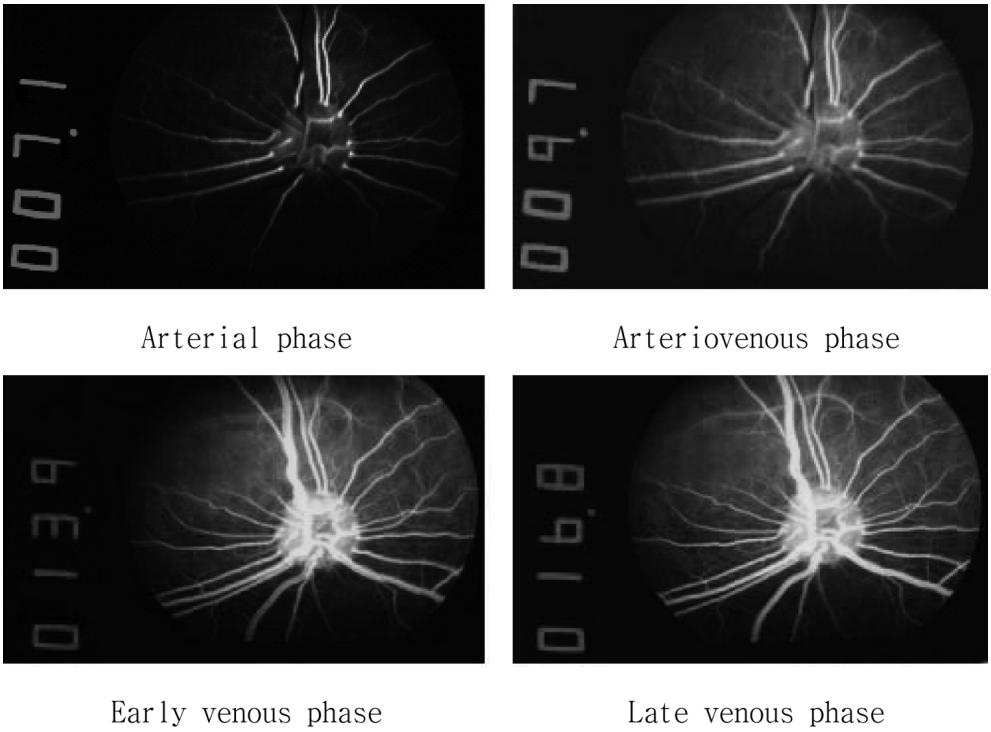

Fig 1. Representative images of the retinal angiographic phase in a dog.

Table 1. Fluorescein angiographic onset times of the injectable anesthetics in dogs

Group	Angiographic phases (sec)			
	AP	AVP	EVP	LVP
AK	7.20 ± 1.13[a]*	10.6 ± 1.47[a]	14.43 ± 2.16[a]	19.14 ± 2.94[a]
XK	7.40 ± 0.65[a]	10.4 ± 1.13[a]	13.78 ± 1.25[a]	18.03 ± 1.38[ab]
DK	5.65 ± 0.77[b]	8.48 ± 1.20[b]	11.40 ± 1.52[b]	15.73 ± 1.63[bc]
ZT	5.35 ± 0.75[b]	7.63 ± 0.66[b]	9.80 ± 1.20[b]	13.73 ± 0.91[c]

AK group : acepromazine - ketamine group, XK group : xylazine - ketamine group, DK group : diazepam - ketmaine group, ZT group : zolazepam - tiletamine group. ; AP : arterial phase, AVP : arteriovenous phase, EVP : early venous phase, LVP : late venous phase.
* Data is expressed as the mean ± SD (n=8).
[a,b,c] Different superscript within the same column means statistically different at $p<0.05$.

Table 2. Fluorescein angiographic duration of the injectable anesthetics in dogs

Group	Durations (sec)		
	D_1	D_2	D_3
AK	3.41 ± 0.72[b]*	3.81 ± 1.03[b]	4.71 ± 1.08[a]
XK	3.01 ± 0.59[ab]	3.36 ± 1.05[ab]	4.25 ± 0.91[a]
DK	2.83 ± 0.69[ab]	2.93 ± 0.85[ab]	4.33 ± 0.70[a]
ZT	2.28 ± 0.39[a]	2.18 ± 0.61[a]	3.93 ± 0.66[a]

AK group : acepromazine - ketamine group, XK group : xylazine - ketamine group, DK group : diazepam - ketmaine group, ZT group : zolazepam - tiletamine group. ; D_1 : between arterial phase and arteriovenous phase, D_2 : between arteriovenous phase and early venous phase, D_3 : between early venous phase and late venous phase.
* Data is expressed as the mean ± SD (n=8).
[a,b] Different superscript within the same column means statistically different at $p<0.05$.

after administering the anesthetics in the AK and XT groups (Fig 4 - 6). The rectal temperature did not change significantly except in the ZT group (Fig 7).

The heart rates showed significant relationship with the AP, AVP, EVP, LVP, D_1 and D_2. The systolic arterial pressure was associated with the EVP, LVP and D_3.

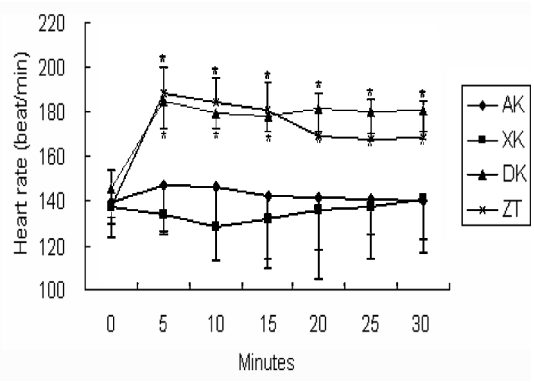

Fig 2. Changes in the mean heart rates after administering anesthetics in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

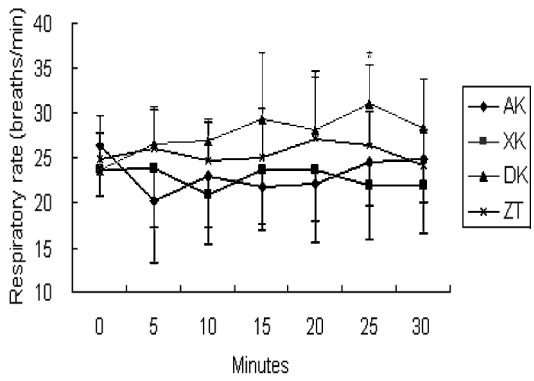

Fig 3. Changes in the respiratory rates after administering the anesthetics in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

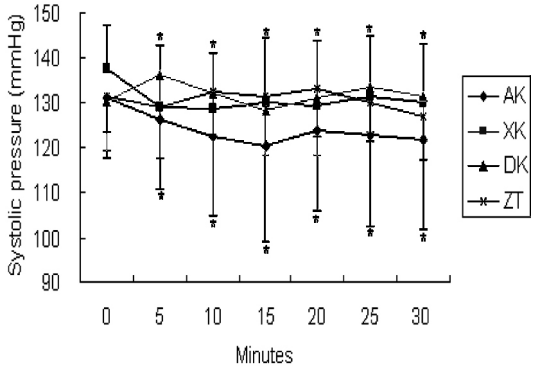

Fig 4. Changes in the systolic arterial pressure after administering the anesthetics in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

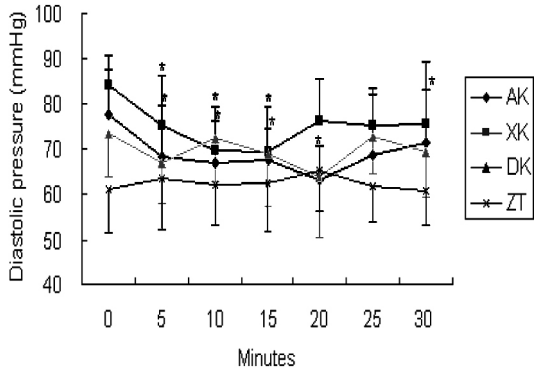

Fig 5. Changes in the diastolic arterial pressure administering the anesthetics in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

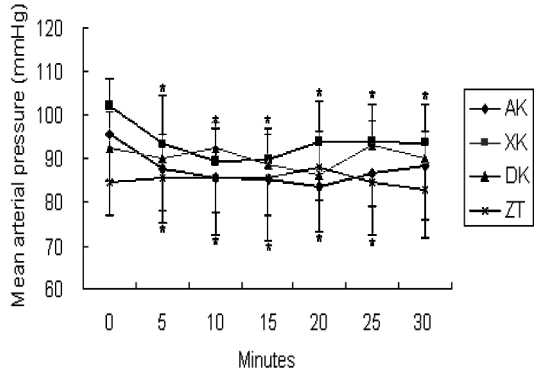

Fig 6. Changes in the mean arterial pressure after administering the anesthetics administering the in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

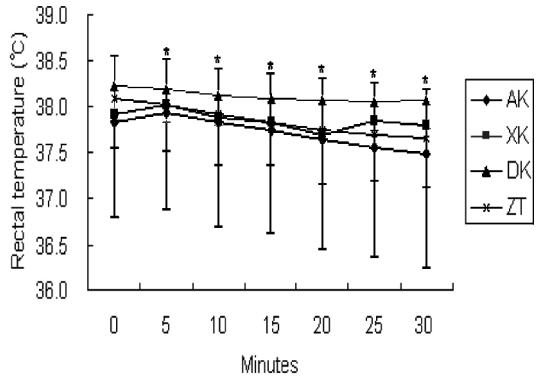

Fig 7. Changes in the rectal temperatures after administering the anesthetics in dogs. AK: acepromazine - ketamine, XK: xylazine - ketamine, DK: diazepam - ketmaine, ZT: zolazepam - tiletamine.
* Significant difference after administering the injectable anesthetics within the group; Paired t-test (* $p<0.05$).

Clinical evaluation

Table 3 summarizes the clinical evaluation. The DK and ZT groups showed more interference by the angiographic interruption factors than the AK and DK groups. Eye dropping and nystagmus occurred in the AK group, but only case of one eye dropping was observed in the XK group. Nystagmus, head movement and tongue movement were observed in one dog each in the DK group. Nystagmus and tongue movement were noted in two dogs in the ZT group. No third eyelid protrusion was recorded. There was no significant relationship between effects of the injectable anesthetics and the clinical signs.

Discussion

Fluorescein angiography is a diagnostic method for detecting chorioretinal vascular changes(17). The doses of the sodium fluorescein dye used vary widely; 12 mg/kg (11), 15 mg/kg (19) and 25 mg/kg (4). Dogs were given 50 mg/kg fluorescein dye showed intense fluorescein. Higher doses of the fluorescein dye had a greater possibility of entering the aqueous and vitreous humor and obscuring the ocular fundus (4). With lower doses (10 and 15 mg/kg), at 10 minutes after the injection, fluorescein could be detected only in the nontapetal fundus with no fluorescein detected in the tapetal fundus (4).

The axial eye position is important for examining fluorescein angiography. Martin *et al*. (13) used conjunctival forceps to maintain the axial eye position. Schaepdrijver *et al*. (16) used sutures inserted transconjunctivally under the tendons of the dorsal rectus muscle, ventral rectus muscle, and medial rectus muscle to adjust the eyeball position. However, these techniques can be invasive. Therefore, in this study, an eye speculum was used instead of forceps and suturing. Placing the eye speculum was helpful in not only satisfactorily restraining the eyeball in the axial position but also in preventing third eyelid protrusion.

There are different opinions regarding the appearance of the vein. Martin *et al*. (13) compared the effects of sevoflurane, isoflurane and halothane anesthesia on the fluorescein angiographic phases of dogs. They classified each phase as the arterial phase, the arteriovenous phase (when laminar flow of the dye appeared in the vein) and venous phase (when the veins were totally filled). The onset time of each phase was 14.3 ± 1.9, 18.8 ± 3.3 and 26.0 ± 4.1 in the isoflurane group, 14.4 ± 4.8, 20.5 ± 4.3 and 29.6 ± 5.1 in the sevoflurane group and 22.4 ± 6.6, 27.4 ± 7.9 and 36.1 ± 8.3 in the halothane group, respectively (mean ± SD seconds). Compared with the present study, the onset time and duration of each phase were later and longer under inhalation anesthesia than under injectable anesthesia. Schaepdrijver *et al*. (16) performed a comparative study of fluorescein angiography under the propofol and ketamine anesthesia. The classification used was the arterial phase, arteriovenous phase (when the dye appeared in the retinal capillaries), early venous phase (when the laminar flow of dye appeared in the veins) and late venous phase (when the veins were totally filled with the dye). The onset time of each phase was 7.0 ± 0.8, 8.4 ± 1.2, 9.4 ± 1.6 and 13.5 ± 2.1 in the propofol group and 9.1 ± 2.1, 10.9 ± 3.0, 11.4 ± 3.0 and 15.6 ± 3.5 in the ketamine group, individually (mean ± SD seconds). Compared with the present study, the onset time of the arterial phases in the propofol group was similar to that of the AK and XK groups. However, it was slower than that of the DK and ZT groups. The arterial phasic onset time in the ketamine group was slower than in all groups in this study. The total duration of each phase was approximately 4 seconds shorter than those of the present study. The onset of each phase might have been influenced by the anesthetic technique used. Therefore, they do not precisely indicate the patient's status (13). For this reason, the start time of the various angiographic phases should not be considered as being an indicative value (16).

There were significant differences in all angiography variables between the groups except for D_3 (total duration of the venous phase). A decision of the late venous phase might be subjective because the phase was characterized by a homogeneous and completing filling of the primary venules.

The total duration of the angiographic phases were longer in the AK and XK groups than in the DK and ZT groups. The photographs are taken at a rate of 1 record per 0.7 - 1 seconds for fluorescein angiography (9). A too rapid passage of the fluorescein dye may have an adverse effect on the imaging, which would impede accurate measurements of the onset time of each phase.

Ketamine has some emergence reactions, which are char-

Table 3. Effects of the injectable anesthetics on the angiographic interruptible factors during fluorescein angiography in dogs

Group	Angiographic interruptible factors				
	Eyeball dropping	3rd eyelid protrusion	Nystagmus	Head movement	Tongue movement
AK	1 / 8*	0 / 8	1 / 8	0 / 8	0 / 8
XK	1 / 8	0 / 8	0 / 8	0 / 8	0 / 8
DK	0 / 8	0 / 8	1 / 8	1 / 8	1 / 8
ZT	0 / 8	0 / 8	2 / 8	0 / 8	2 / 8

AK group : acepromazine - ketamine group, XK group : xylazine - ketamine group,
DK group : diazepam - ketmaine group, ZT group : zolazepam - tiletamine group.
* Number of incidence / total number of dogs.

acterized by ataxia, increased motor activity, hyperreflexia, sensitivity to touch, avoidance behavior of invisible objects, cardiovascular stimulating effects, and some times violent recovery (21). Premedication or the concurrent administration of α_2-agonists, acetylpromazine, or a benzodiazepine derivative decreases the incidence of these adverse emergence reactions (5, 6, 7, 12). Benzodiazepines and ketamine induce a significant increase in heart rate as well as mild respiratory depression (12). Tachycardia and transient hypotension were reported to occur after injecting 10 or 20 mg/kg zolazepam - tiletamine combination (14). An intravenous injection of 6.6, 13.2 and 19.8 mg/kg zolazepam - tiletamine combination caused an increase in the heart rate (8, 12). The DK and ZT groups had a higher heart rate than the AK and DK groups. There was significant relationship between the heart rate and onset time of each phase in this study.

The critical fact for fluorescein angiography is to maintain the eye in a central position and with no incidence of enophalmos or third eyelid protrusion (1). It is essential to maintain a calm restraint without interference for at least 30 seconds initially (20). The AK and XK groups showed relatively less interference by the angiographic interruption factor compared with the DK and ZT groups in this study.

Nausea, vomiting, urticaria, skin necrosis with dye extravasation have all been reported (10). Fatal anaphylactic shock can occur during fluorescein angiography, albeit rarely (2). No adverse effects were encountered in this study period except for fluorescein urine, which decreased within 24 hours in all dogs.

There were significant differences on each angiographic onset time and duration depending on the changes in the heart rate and arterial pressure ($p<0.05$). The AK and XK groups showed a long angiographic duration, which allowed an accurate evaluation, and there was less interference from the angiographic interruption factors. Overall, AK and XK are more useful for performing fluorescein retinal angiography than DK and ZT.

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