Original Article

Effects of systemic administrations of medetomidine and xylazine on ex vivo platelet aggregation in clinically normal cats

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Abstract

This study aimed to investigate and compare the effects of systemic administration of medetomidine and xylazine on ex vivo platelet aggregation in clinically normal cats. Five cats were repeatedly used in each of 7 groups. The cats received saline as the non-medicated control; 0.5, 2.0, and 4.0 mg/kg xylazine; and 20, 80, and 160 µg/kg BW medetomidine intramuscularly. Venous blood was collected 4 times (0, 2, 4, and 7 h) after injection of both agents and platelet-rich plasma was prepared. Ex vivo percent platelet aggregation was determined via a turbidimetric method. Collagen and adenosine diphosphate (ADP) were used to initiate aggregation. Administrations of xylazine at all dosages did not significantly change the platelet aggregation induced by ADP and collagen compared with the control. In contrast, administration of medetomidine at 80 µg/kg significantly reduced platelet aggregation induced by both ADP and collagen compared with the control, whereas medetomidine at the other dosages did not significantly change the platelet aggregation induced by ADP and collagen. These results indicate that systemic administration of xylazine can be used in feline practice without concern for adverse effects on platelet function, although, if medetomidine is used, even in limited dosages, it may inhibit platelet aggregation.

Keywords: cats, ex vivo, intramuscular administration, medetomidine, platelet aggregation, xylazine

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Introduction

In practice, the α_2-adrenoceptor agonists medetomidine (MED) and xylazine (XYL) are widely used to produce reliable sedation, analgesia and muscle relaxation in cats (Lemke, 2004). Although both drugs are used similarly in practice, there are differences between them. MED is a more potent, selective and specific α_2-adrenoceptor agonist compared with XYL. The ratio of α_2-adrenoceptor selectivity to α_1-adrenoceptor selectivity of MED (1,620:1) is approximately 10-fold as great as that of XYL (160:1) (Virtanen, 1989). In addition, MED, in contrast to XYL, has an imidazole (I) ring that has an affinity for I-receptors (Murrell and Hellebrekers, 2005).

In cats, overactivity of the sympathetic nervous system and increased catecholamine concentrations are induced in conditions such as pheochromocytoma (Wimpole *et al*., 2010), endotoxin shock (Feuerstein *et al*., 1981) and acute stress (Rand *et al*., 2002). Hypercatecholaminemia reportedly has an influence on hemostasis, including disseminated intravascular coagulation (Barrera *et al*., 2013) and thromboembolism (Chun *et al*., 1997; Squizzato *et al*., 2007; DeClue *et al*., 2009), by acting on platelets. Because both MED and XYL reduce plasma concentrations of adrenaline and noradrenaline in cats (Kanda and Hikasa, 2008), it may be important on blood homeostasis to examine the platelet response in cats administered systemically with MED or XYL.

Regarding in vitro platelet responses to catecholamines in small animals, adrenaline alone does not induce platelet aggregation; however, it potentiates platelet aggregation stimulated by other platelet agonists including ADP, collagen and thrombin in dogs (Hikasa *et al*., 1999), cats (Hart and Nolte,1991; Matsukawa and Hikasa, 2020) and rabbits (Yokota *et al*., 2013). It has been reported that adrenaline-potentiated platelet aggregation is mediated by α_2-adrenoceptors on platelets because this is blocked by α_2-adrenoceptor antagonists but not by α_1-adrenoceptor antagonists in dogs (Hikasa *et al*., 1999), cats (Matsukawa and Hikasa, 2020) and rabbits (Yokota e*t al*., 2013), as well as in humans (Pinthong *et al*., 2004). However, it has been reported that many I α_2-adrenoceptor agents inhibit adrenaline-potentiated platelet aggregation in dogs (Hikasa *et al*., 1999), cats (Matsukawa and Hikasa, 2020, and rabbits (Yokota *et al*., 2013). Nonadrenergic I_1 and I_2 receptors that are pharmacologically distinct from α_2-adrenoceptors have been expressed in canine and feline platelets (Hikasa *et al*., 2013), as well as in human platelets (Piletz and Sletten, 1993; Piletz *et al*., 1996). In addition, the densities of I_1 and I_2 receptors and α_2-adrenoceptors differ among animal species (Hikasa *et al*., 2013). MED binds to I_1 and I_2 receptors on feline platelets whereas XYL does not have an affinity for I_1 and I_2 receptors (Hikasa *et al*., 2013). A recent in vitro study has reported that medetomidine inhibited adrenaline-potentiated platelet aggregation induced by ADP or collagen in a dose-dependent manner but XYL was ineffective in inhibiting adrenaline-potentiated aggregation (Matsukawa and Hikasa, 2020). However, to the best of our knowledge, there are no published reports on the blood platelet aggregation in cats that were administered XYL or MED systemically. Therefore, this study was conducted to compare the effects of MED and XYL administered intramuscularly (IM), on ex vivo platelet aggregation in healthy cats.

Materials and Methods

Animals: Two healthy male (2 neutered) and 3 healthy female (2 neutered) adult mixed-breed cats with a mean age of 9.2 years (standard deviation [SD] = 3.3) and a mean weight of 3.8 kg (SD = 0.56) were used in this study. They were fed a standard commercial dry food formulated for cats and raised in a laboratory with appropriate animal management facilities. Physical examination and hematological analysis prior to the experiments revealed that all the cats were clinically normal. The animals' signalments, some hematological, and blood biochemical profiles are summarized in Table 1. The study protocol was approved by the Animal Research Committee of Tottori University (approval no. 12-T-25).

Experimental procedures: Five cats were used repeatedly for 7 treatment groups (5 cats per group) in a modified randomized design. In group 1, each cat was administered a physiological saline solution (0.1 ml/kg) IM as the non-medicated control. In groups 2, 3, 4, 5, 6, and 7, each cat received IM 0.5, 2.0, and 4.0 mg/kg XYL hydrochloride (Sigma-Aldrich Japan K.K., Tokyo, Japan), and 20, 80, and 160 μg/kg MED hydrochloride (Dorbene, Syva Laboratorios, S.A., Spain), respectively. Seven groups were denoted as controls, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160. Cat-1 was treated with control, XYL-0.5, MED-20, XYL-2, MED-80, XYL-4, and MED-160 in that order. Cat-2 was treated in the order of XYL-0.5, MED-20, XYL-2, MED-80, XYL-4, MED-160, and control. Cat-3 was treated in the order of MED-20, XYL-2, MED-80, XYL-4, MED-160, control, and XYL-0.5. Cat-4 was treated in the order of XYL-2, MED-80, XYL-4, MED-160, control, XYL-0.5, and MED-20. Cat-5 was treated in the order of MED-80, XYL-4, MED-160, control, XYL-0.5, MED-20, and XYL-2.

Intervals between treatments ranged from 1 to 4 weeks for each cat in this study. The intervals were 1 to 2 weeks after control, lowest and middle-dose MED or XYL treatments, and 2 to 4 weeks after the highest-dose MED or XYL treatments. The washout period (mean ± SD, week) between treatments was 2.3 ± 1.0 in Cat-1, 2.5 ± 0.8 in Cat-2, 2.3 ± 1.0 in Cat-3, 2.5 ± 1.2 in Cat-4, 2.3 ± 0.5 in Cat-5, respectively. The interval of 1 week between treatments was set three times only, once in 3 cats in this study. In each case, the 1-week interval was only between the control and XYL-0.5 treatments.

Food and water were withheld for 12 h before the start of each experiment. Food and water were provided after sample collection at 8 h after injection. The experiments were done in a room where the room temperature was maintained at 25°C.

Blood sampling and preparation of citrated platelet plasma: Jugular blood samples (4.5 ml) were collected in plastic syringes containing 3.2% sodium citrate

solution at a ratio of 1 part anticoagulant to 9 parts blood, 4 times (immediately before injection of the treatment [0 h; baseline] and 2, 4, and 7 h after injection) from each cat. The citrated blood was centrifuged at 90 to 110×*g* for 10 to 15 mins to obtain platelet-rich plasma (PRP). The platelet-poor plasma (PPP) was obtained by centrifuging the remaining citrated blood after collecting PRP at 1,500×*g* for 15 mins. The final platelet count in PRP was adjusted to 25 to 30 × 10^4 platelets/μL via dilution with autologous PPP.

The determination of time points of measurement is involved with the technical aspects of measuring platelet aggregation, since the aggregation test must be performed immediately after blood sampling. It takes approximately 1 to 1.5 h to complete the preparation of citrated platelet plasma and the platelet aggregation experiment after one blood sampling. So, we chose an interval of at least 2 h before the next blood sampling. In addition, after administration of the highest doses of MED and XYL, the cat was deeply sedated at 2 h and sedation was continued for 4 h and disappeared at 7 h. For these reasons, we decided 4 time points of the measurement at 0, 2, 4 and 7 h after injection.

Table 1 Summary of signalments, hematologic and blood biochemical profiles in 5 cats used in this study.

Cat	1	2	3	4	5	Reference range
Breed	Mixed	Mixed	Mixed	Mixed	Mixed	
Gender	Female intact	Female ovariohysterectomized	Female ovariohysterectomized	Male castrated	Male castrated	
Age (year)	12	11	8	11	4	
Body weight (kg)	3.1	4.3	3.4	3.8	4.4	
Clinical signs	Healthy	Healthy	Healthy	Healthy	Healthy	
Packed cell volume (%)	32	34	40	38	42	30-45
Red blood cell counts (×10^4/mm^3)	623	631	865	754	889	500-1,000
White blood cell counts (×10^2/mm^3)	123	107	119	116	98	55-195
Platelet counts (×10^4/mm^3)	45	41	39	33	56	30-80
Plasma protein (mg/dL)	7.4	6.7	6.8	7.3	7.2	5.7-7.8
Blood glucose (mg/dL)	89	109	113	90	94	71-148
Total cholesterol (mg/dL)	166	109	99	121	140	89-176
Aspartate aminotransferase (IU/L)	48	32	36	40	26	18-51
Alanine aminotransferase (IU/L)	79	61	60	57	41	22-84
Blood urea nitrogen (mg/dL)	30	26	25	30	21	17.6-32.8
Creatinine (mg/dL)	1.5	1.4	1.1	1.3	1.3	0.8-1.8

Aggregation experiments: The platelet aggregation experiments were performed as previously described (Hikasa *et al*., 1999; Yokota *et al*., 2013). Briefly, a turbidimetric method was used. The percent aggregation was determined after adding the aggregation agent and was standardized via the assumption that PPP and PRP represented 100% and 0% light transmission, respectively. In each PRP sample, the aggregation effects of ADP and collagen were examined as follows. An aliquot (200 μL) of PRP was placed in an aggregometer (MCM Hema Tracer 804, LMS Co Ltd, Tokyo, Japan) at 37°C, and 1 min later, an aliquot (22 μL) of ADP (0, 1, 3, and 5 μmol/L) or collagen (0, 1, 3, and 5 μg/ml) was added to the PRP and the maximum percentage aggregation was recorded during the subsequent 10-min interval.

Statistical analysis: Statistical analysis was performed using commercially available statistical programs (Prism 7.0, GraphPad Software Inc, San Diego, CA). Data was reported as the mean ± standard error (SE). To determine the potency of the platelet aggregatory effect of ADP or collagen, the mean effective dose (ED) 50 that caused 50% aggregation was obtained from the concentration-response curve on platelet aggregation. The ED50 and percent aggregation data were assessed for normality of distribution with the Shapiro-Wilk test. When the data was normally distributed, the paired *t* test was used for comparison between the groups at 0, 2, 4 and 7 h after injection of XYL or MED. When the data was not normally distributed, the Wilcoxon-Mann-Whitney test was used to determine significant differences. The paired *t* test was used to determine significant differences for change in percentage aggregation that were expressed as a percentage of the value for the time 0 h (baseline), which was assigned a value of 100%. For all tests, differences were considered significant at values of $P < 0.05$.

Results

ADP-induced platelet aggregation response: In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80 and MED-160 groups, 3 μmol/L ADP-induced maximum platelet aggregations were 76.2 ± 6.2%, 78.0 ± 3.4%, 80.2 ± 5.1%, 79.6 ± 0.9%, 78.0 ± 3.5%, 81.8 ± 2.4% and 79.4 ± 3.1% before XYL or MED injection (0 h; baseline), respectively. There were no significant differences in the maximum aggregation between the groups at the baseline. In the control and all of the XYL groups, no significant differences for change in percentage aggregation, which was expressed as a percentage of the value for the baseline, were observed at 2, 4 and 7

h after saline or XYL injection (Fig. 1A). In addition, there were no significant differences in percentage aggregation between XYL and control groups at 2, 4 and 7 h. In contrast, a significant decrease in the percentage aggregation was observed at 2, 4 and 7 h compared with the baseline in the MED-80 group but not in the MED-20 and MED-160 groups (Fig. 1B). The percentage aggregation was significantly lower in the MED-80 group than in control group at 2, 4, and 7 h. There were no significant differences in percentage aggregation between MED-20 and the control groups and between MED-160 and the control groups at any time.

The maximum platelet aggregation induced by 5 µmol/L ADP was 84.0 ± 3.1%, 76.8 ± 3.8%, 86.8 ± 2.0%, 81.2 ± 1.0%, 79.2 ± 2.6%, 83.2 ± 2.0%, and 82.6 ± 3.8% at the baseline in the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80 and MED-160 groups, respectively. There were no significant differences in the maximum aggregation between the groups at the baseline. Aggregation responses induced by 5 µmol/L ADP after XYL or MED injection were similar to those by 3 µmol/L ADP. In the control and all of the XYL groups, no significant changes in percentage aggregation were observed at any time after saline or XYL injection (Fig. 2A). In the MED-80 group, the aggregation induced by 5 µmol/L ADP decreased at 2, 4, and 7 h compared with the baseline after MED injection (Fig. 2B). The percentage aggregation was significantly lower in the MED-80 group than in the control group at 2, 4 and 7 h. In the MED-20 and MED-160 groups, the aggregation induced by 5 µmol/L ADP did not significantly decrease at 2–7 h compared with the baseline after MED injection (Fig. 2B).

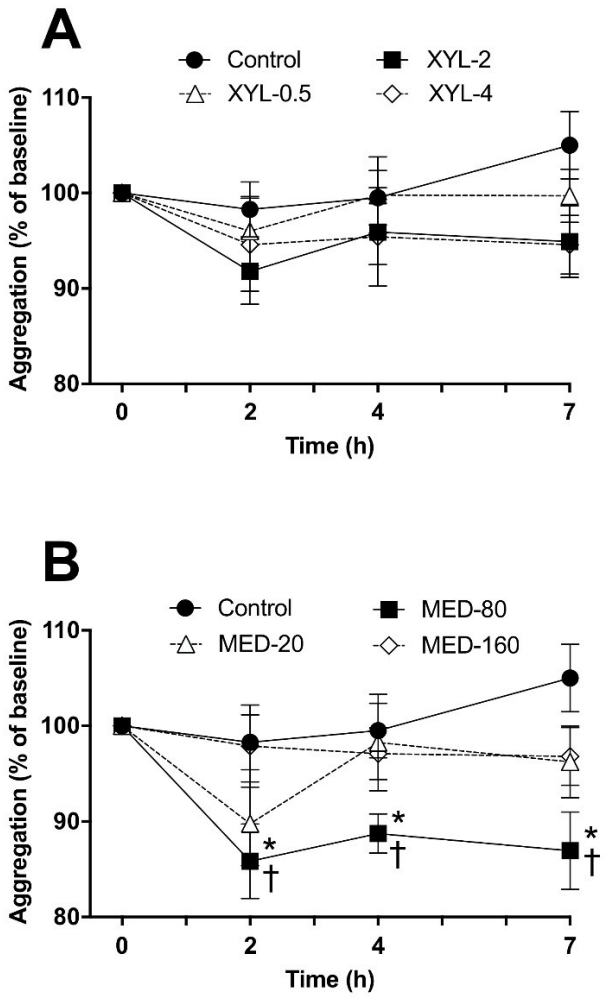

Figure 1 Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 3.0 µmol/L adenosine diphosphate (ADP) after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of ADP at 0 h was assigned a value of 100%). Control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups showed physiological saline solution, 0.5, 2.0, 4.0 mg/kg xylazine, 20, 80, and 160 µg/kg medetomidine, respectively. *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group.

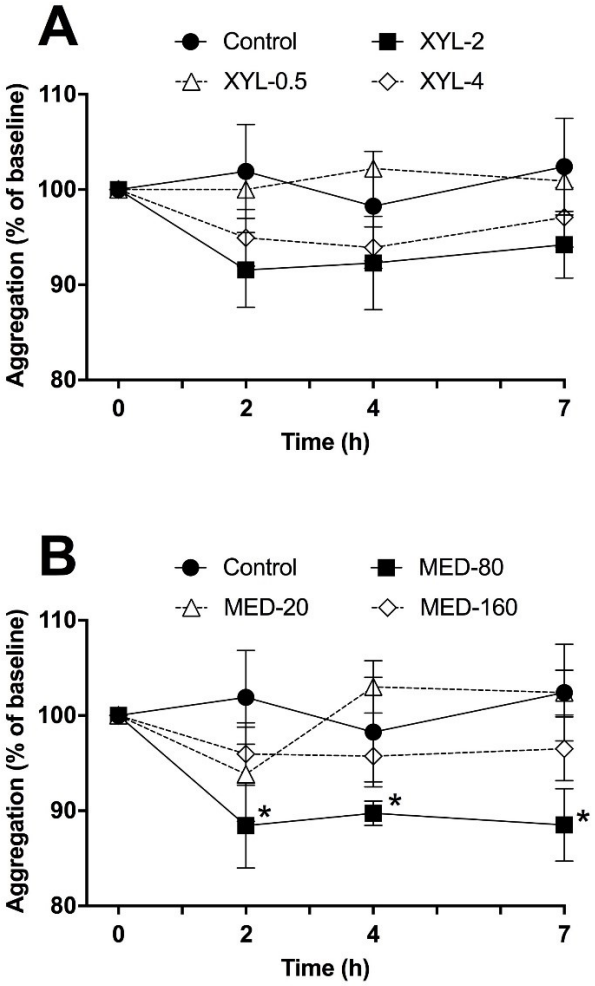

Figure 2 Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 5.0 µmol/L adenosine diphosphate (ADP) after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of ADP at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. See Figure 1 for the remainder of the key.

The ED50 values of ADP that caused 50% platelet aggregation in all groups are summarized in Table 2. The ED50 of ADP in the MED-80 group was significantly higher at 2–7 h than at the baseline. In addition, the ED50 value of ADP in the MED-80 group at 2 h was significantly higher than that of the control, XYL-2, and XYL-4 groups. There were no significant differences in ED50 values between the other groups although the ED50 value in MED-20 and MED-160 tended to be higher than that of the control at 2 h and 7 h, respectively.

Collagen-induced platelet aggregation response: In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80, and MED-160 groups, 3 µg/ml collagen-induced maximum platelet aggregation at the baseline was 77.6 ± 6.4%, 80.8 ± 3.8%, 81.8 ± 5.9%, 84.0 ± 2.0%, 81.6 ± 4.7%, 83.4 ± 2.9%, and 84.0 ± 3.2%, respectively. There were no significant differences in the aggregations between the groups at the baseline. In the control and all of the XYL groups, 3 µg/ml collagen-induced aggregation did not significantly change at 2, 4 and 7 h after saline or XYL injection compared with the baseline (Fig. 3A). In contrast, a significant decrease in the percentage aggregation was observed at 2 and 4 h compared with the baseline in the MED-80 group but not in the MED-20 and MED-160 groups (Fig. 3B). The percentage aggregation was significantly lower in MED-80 than in the control at 2 h but there were no significant differences between XYL and the control groups or and between MED-20 or MED-160 and the control groups at any time.

In the control, XYL-0.5, XYL-2, XYL-4, MED-20, MED-80 and MED-160 groups, 5 µg/ml collagen-induced maximum platelet aggregation at the baseline was 79.2 ± 7.7%, 81.2 ± 3.9%, 83.4 ± 5.7%, 84.2 ± 2.3%, 80.8 ± 4.1%, 84.8 ± 3.3%, and 84.0 ± 4.4%, respectively. There were no significant differences in the aggregations between the groups at the baseline. In the control and all of the XYL groups, 5 µg/ml collagen-induced aggregation did not significantly change at 2, 4 and 7 h after saline or XYL injection compared with the baseline (Fig. 4A). In contrast, a significant decrease in the percentage aggregation was observed at 2 and 4 h compared with the baseline in the MED-80 group but not in the MED-20 and MED-160 groups (Fig. 4B). The percentage aggregation was significantly lower in MED-80 than control at 2 h but there were no significant differences between XYL and the control

groups and between MED-20 or MED-160 and the control groups at any time.

The ED50 values of collagen that caused 50% platelet aggregation in all groups are summarized in Table 3. The ED50 of collagen in the MED-80 group was significantly higher at 2 and 4 h than at the baseline. In addition, the ED50 value of collagen in the MED-80 group at 2 h was significantly higher than that of the control and the MED-160 groups. There were no significant differences in ED50 value between the other groups although ED50 value in XYL-4 tended to be higher than that of the control at 2 h.

Table 2 Mean ± standard error (SE) mean effective dose (ED) 50 (ED50) of adenosine diphosphate (ADP) that caused 50% aggregation on ex vivo platelet aggregation in 5 cats administered xylazine or medetomidine intramuscularly

Group	Time (h)											
	0			2			4			7		
Control	1.65	±	0.25	1.63	±	0.20	1.63	±	0.21	1.43	±	0.17
XYL-0.5	1.87	±	0.20	1.66	±	0.21	1.72	±	0.15	1.70	±	0.14
XYL-2	1.81	±	0.29	1.47	±	0.28	1.68	±	0.16	1.50	±	0.22
XYL-4	1.26	±	0.33	1.49	±	0.23	1.42	±	0.29	1.60	±	0.22
MED-20	1.92	±	0.17	2.24	±	0.34	1.52	±	0.22	1.81	±	0.12
MED-80	1.70	±	0.13	2.45	±	0.25*†§‡	2.07	±	0.18*	2.22	±	0.15*
MED-160	1.42	±	0.30	1.52	±	0.15	1.77	±	0.17	1.86	±	0.11

* $P < 0.05$, significantly different from 0 h (baseline).
† $P < 0.05$, significantly different from control.
§ $P < 0.05$, significantly different from XYL-2.
‡ $P < 0.05$, significantly different from XYL-4.

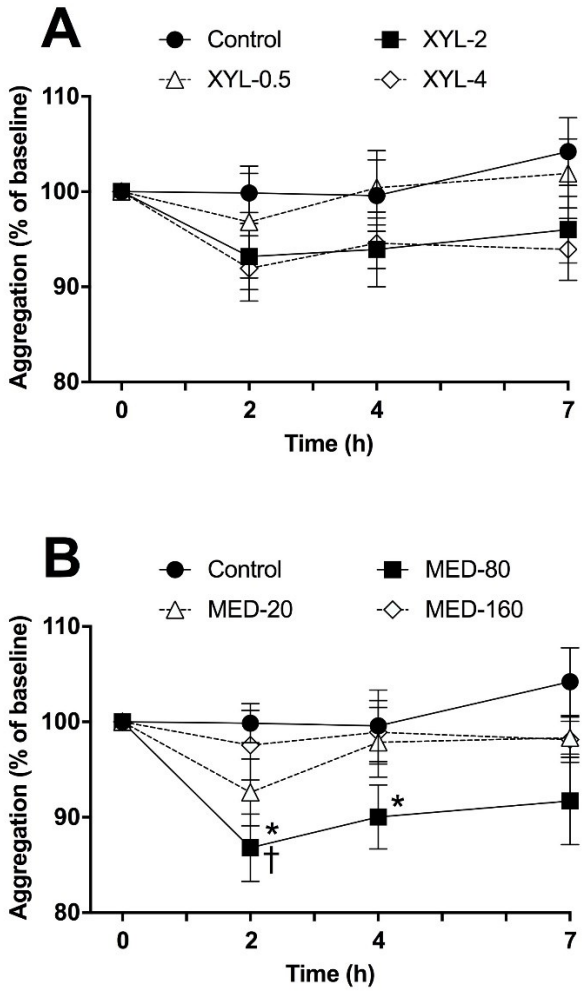

Figure 3 Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 3.0 μg/ml collagen after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of adenosine diphosphate [ADP] at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group. See Figure 1 for the remainder of the key.

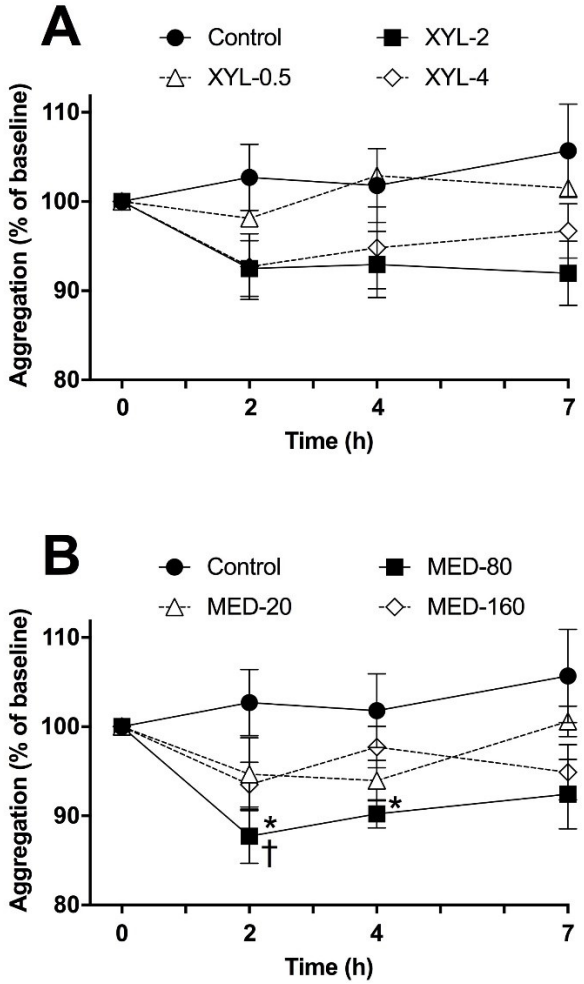

Figure 4 Mean ± standard error (SE) percentage changes of ex vivo platelet aggregation induced by 5.0 µg/ml collagen after an intramuscular administration of xylazine (A) and medetomidine (B) in 5 cats. Values are reported as a percentage of the value for the 0 h (percentage aggregation of adenosine diphosphate [ADP] at 0 h was assigned a value of 100%). *Value differs significantly ($P < 0.05$) from the value for the 0 h. †Value differs significantly ($P < 0.05$) from the value for the control group. See Figure 1 for the remainder of the key.

Table 3 Mean ± standard error (SE) mean effective dose (ED) 50 (ED50) of collagen that caused 50% aggregation on ex vivo platelet aggregation in 5 cats administered xylazine or medetomidine intramuscularly

Group	Time (h)											
	0			2			4			7		
Control	1.61	±	0.37	1.86	±	0.11	2.20	±	0.38	2.00	±	0.30
XYL-0.5	1.69	±	0.38	2.03	±	0.07	1.90	±	0.11	1.88	±	0.10
XYL-2	1.99	±	0.35	1.89	±	0.18	1.84	±	0.16	1.53	±	0.32
XYL-4	1.53	±	0.28	2.13	±	0.25	2.09	±	0.22	1.66	±	0.27
MED-20	1.42	±	0.40	1.72	±	0.60	1.47	±	0.44	1.43	±	0.39
MED-80	1.83	±	0.11	2.48	±	0.23*†§	2.27	±	0.21*	2.14	±	0.28
MED-160	1.50	±	0.19	1.60	±	0.16	1.54	±	0.33	1.89	±	0.17

* $P < 0.05$, significantly different from 0 h (baseline).
† $P < 0.05$, significantly different from control.
§ $P < 0.05$, significantly different from MED-160.

Discussion

The results of this study demonstrate that an IM administration of 80 µg/kg MED inhibited ex vivo platelet aggregation, induced by both 3–5 µmol/L ADP and 3–5 µg/ml collagen in healthy cats; however, the inhibitory effects of MED were not dose-dependent within the tested doses. In addition, this study revealed that IM administrations of 0.5–4 mg/kg XYL did not significantly affect ex vivo platelet aggregation induced by both ADP and collagen. To the best of our knowledge, these findings are the first report outlining the ex vivo platelet aggregatory responses in cats that have received MED and XYL systemically and this is the first study comparing these 2 drugs.

These differences in platelet responses between MED and XYL administrations may be due to differences in receptor selectivity and specificity between the 2 drugs. In a previous in vitro study, it was reported that MED inhibited adrenaline-potentiated aggregation induced by ADP or collagen, whereas XYL was ineffective in inhibiting the adrenaline-potentiated

aggregation in feline platelets (Matsukawa and Hikasa, 2020). It has been demonstrated that both feline and canine platelets have non-adrenergic I_1-receptors labeled by tritiated clonidine and I_2-receptors that had been labeled by tritiated idazoxan as well as α_2-adrenoceptors (Hikasa *et al*., 2013). Furthermore, the affinities of MED to canine platelet I_1- and I_2-receptors have been reported to be approximately 16- and 55-fold, respectively, greater than those of XYL (Hikasa *et al*., 2013), although in cats the affinities of both drugs to platelet I_1- and I_2-receptors have not been reported. A comparative study of the effects of I α-adrenergic agents on intraplatelet cyclic adenosine monophosphate (cAMP) and thromboxane B_2 (TXB_2) in canine and leporine platelets suggested that I α_2-adrenergic agents suppress cAMP production via the α_2-adrenoceptor, while exerting a negative control on TXB_2 generation via the arachidonic acid–thromboxane A_2 pathway (Yokota and Hikasa, 2015). In addition, a previous study has demonstrated that α_2-adrenoceptors are expressed on canine and feline platelets but not on bovine and equine platelets and that all of the 4 animal species platelets have both I_1- and I_2-receptor subtypes (Hikasa *et al*., 2013). It has also been reported that bovine and equine platelets are unresponsive to catecholamines, but certain I α-adrenergic agents inhibit bovine and equine platelet aggregation induced by ADP or collagen (Yokota *et al*., 2013). These findings suggest that I α-adrenergic agents can inhibit platelet aggregation via non-adrenergic receptors including I-receptors. Therefore, the decrease of ex vivo ADP and collagen-induced platelet aggregation by the administration of 80 µg/kg MED in this study may be due to inhibiting platelet aggregation via the non-adrenoceptor binding sites including I_1- and I_2-receptors on feline platelets.

On the other hand, in the present study, 4 mg/kg XYL and 20 µg/kg MED insignificantly tended to reduce ex vivo collagen- or ADP-induced platelet aggregation at 2 h after administration. Both adrenaline and noradrenaline are reported to enhance the platelet aggregation induced by other stimulants, including ADP and collagen in cats (Hart and Nolte,1991; Matsukawa and Hikasa, 2020). Administrations of MED and XYL at the tested dosages in this study have been reported to reduce plasma concentrations of adrenaline and noradrenaline in cats (Kanda and Hikasa, 2008). These reports suggest that the decrease or the declining trend of platelet aggregation following administration of MED and XYL in this study may be partially related to the inhibition of endogenous catecholamine secretion via α_2-adrenoceptors, accompanied by the sedative effects of both agents. However, this reason alone cannot explain the difference in the effects of MED and XYL on ex vivo platelet aggregation.

In the present study, in cats, IM administration of MED at 20–160 µg/kg did not inhibit dose-dependent platelet aggregation induced by ADP and collagen. Similar dose-independent effects at higher doses (160 µg/kg) of MED have been also reported on hyperglycemia, hypocatecholaminemia, and diuresis induced by MED in cats (Kanda and Hikasa, 2008; Murahata and Hikasa, 2012) and dogs (Ambrisko and Hikasa, 2002; Talukder and Hikasa, 2009). Clonidine and related drugs may be able to not only inhibit noradrenaline release in the rat cerebral cortex through the α_2-adrenoceptor-mediated mechanism but they may also induce a paradoxical noradrenaline release through an indirect mechanism related to a functional activity on I-receptors (Meana *et al*., 1997). It has been also shown that noradrenaline release is reduced by I_1-receptors in addition to α_2-adrenoceptors in pithed hypertensive rats (Raasch *et al*., 2003), while I_2-receptor selective ligands elevated extrasynaptic noradrenaline release in a rat brain microdialysis study (Abu Ghazaleh *et al*., 2007). These results suggest that I α_2-adrenergic agents at higher concentrations exert complex effects on catecholamine secretion via α_2-adrenoceptors, I_1-receptors, and I_2-receptors. The precise mechanisms by which the higher doses of MED do not further reduce platelet aggregation are not clear. However, as MED has an affinity for both I_1- and I_2-receptors on feline platelets (Hikasa *et al*., 2013), the dose-independency of MED on the inhibitory effect of platelet aggregation may be due to the complicated effects via the I_1- and I_2-receptors or other I-receptor subtypes at a higher concentration of MED.

Both XYL and MED are often used for sedation and analgesia and as a premedication for general anesthesia. The results in this study indicate that XYL may be used in cats with minimal concern for adverse effects on platelet function and hemostasis because, in clinical use, administrations of XYL at recommended doses (0.5–2 mg/kg) do not significantly affect platelet aggregation. However, the use of MED at a limited dose (80 µg/kg) may have inhibitory effects on feline platelet aggregation during certain events such as blood vessel damage and collagen exposure. On the other hand, it has been suggested that sympathetic overactivity and hypercatecholaminemia may influence hemostasis via actions on platelets, coagulation and fibrinolytic factors and endogenous anticoagulants (Squizzato *et al*., 2007). In cats, hypercatecholaminemia occurs in conditions such as pheochromocytoma (Wimpole *et al*., 2010), endotoxin shock (Feuerstein *et al*., 1981) and acute stress (Rand *et al*., 2002). Fatal thromboembolism has been reported in a cat with pheochromocytoma (Chun *et al*., 1997). A low-dose endotoxin infusion induces platelet aggregation (DeClue *et al*., 2009) and intravascular coagulation is manifested during endotoxin shock in cats (Lucas and Kitzmiller, 1972). Therefore, the results of the study reported here suggest that MED may have clinical benefits for the hypercoagulatory state with hypercatecholaminemia because catecholamines have a stimulatory effect on feline platelet aggregation (Matsukawa and Hikasa, 2020). However, further study will be required to examine the effects of MED on platelet aggregation under various pathological conditions in cats.

In conclusion, administration of MED at 80 µg/kg reduced ex vivo platelet aggregation induced by both ADP and collagen compared with the control in this study. Administrations of XYL and MED at other dosages did not significantly affect the aggregation induced by ADP and collagen. Administrations of 4 mg/kg XYL and 20 µg/kg MED insignificantly tended to reduce ex vivo collagen- or ADP-induced platelet aggregation. These findings may be partially related to

the inhibition of endogenous catecholamine secretion via α_2-adrenoceptors accompanied by the sedative effects of both agents. However, the difference in the effects of MED and XYL on ex vivo platelet aggregation could not be explained only by the inhibition of catecholamine secretion. The present results suggest that the I structure, in part, plays a role in the inhibition of platelet aggregation. It was also found that the MED-induced inhibition of platelet aggregation was not dose-dependent in cats. These results indicate that systemic administration of XYL could be used in feline practice without concern for adverse effects on platelet function although, if MED is used, even in limited dosages, it may inhibit platelet aggregation.

Conflict of interest: The authors have no conflict of interest.

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