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Comparative Study of Two Anesthetic Combinations (Zoletil/Midazolam and Zoletil/Xylazine) in Pigs

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Abstract : This study was performed to compare the anesthetic and cardiorespiratory effects of the tiletamine/zolazepam/xylazine (TZX) combination and tiletamine/zolazepam/midazolam (TZM) combination. Eight healthy Landrace X Yorkshire pigs were randomly assigned to two groups. Each group was composed of four pigs. The pigs in group 1 (TZX) received tiletamine/zolazepam (2 mg/kg, IM) and xylazine (2 mg/kg, IM). The pigs in group 2 (TZM) received tiletamine/zolazepam (2 mg/kg, IM) and midazolam (0.5 mg/kg, IV). Induction time, anesthesia time and standing time were recorded for each pig. The scores of anesthetic effects were subjectively evaluated every 15 minutes during anesthesia. Cardiopulmonary parameters (heart rate, arterial blood pressure, respiratory rate and rectal temperature) were monitored and recorded 0, 5, 15, 30, 45 and 60 minutes after administration of drugs. Arterial blood gases (pH_a, P_aCO_2 and P_aO_2) and oxygen saturation (SO_2) were analyzed at same times. The scores of anesthetic effects decreased in the TZX group compare with the TZM group. From 5 to 85 minutes the mean heart rate in the TZX group was significantly lower than those in the TZM group. Mean arterial blood pressure in the TZX group was significantly higher than those in the TZM group at 5, 15 and 30 minutes. Both drug combinations provided a smooth induction and good immobilization. Scores of anesthetic effects in the TZM group were better than those in the TZX group. The effects to the cardiorespiratory function and temperature were lesser in the TZM group than those in the TZX group. In conclusion, when the two drug combinations were compared, the TZM group showed better anesthetic effects and less cardiorespiratory effects.

Key words : anesthesia, midazolam, pig, xylazine, zoletil.

Introduction

The pig is an excellent experimental animal in comparative physiology and has thus been widely used in clinical research (4). The pig is physiologically more closely related to people than are most other species. Because of this similarity, swine plays an important role in human medical research. The cardiopulmonary system and other anatomic features are similar to those of people. Thus, pigs have become widely utilized as laboratory research animal (8). Anesthetic management of pigs can be difficult due to their behavior when physically restrained. Many protocols were developed for suitable anesthesia of pigs.

Tiletamine and zolazepam (TZ) alone induces rapid immobilization but does not produce adequate muscle relaxation and analgesia sufficient for surgery in pigs. Thus, xylazine has been combined to induce effective and safe anesthesia with good muscle relaxation (14).

The benzodiazepines (midazolam) are useful in pigs. Although there are many benzodiazepine derivatives, they produce good effects in pigs. Midazolam induce hypnosis, sedation, and muscle relaxation, but little or no analgesia. Thus, they are combined with an anesthetic or strong analgesic to enhance anesthetic action (10).

The purpose of this study was to evaluate the cardiopulmonary effect of a combination of TZ and midazolam in pigs and to compare its efficacy as an anesthetic with that of TZ and xylazine.

Materials and Methods

Animals

Eight healthy Landrace X Yorkshire pigs, with a mean body weight of 25.3 ± 3.3 kg, were used in this study. Food was withheld for 48 hours, and water was withheld for 12 hours before anesthesia.

Instrumentation

Anesthesia was induced by face mask and maintained with isoflurane (Forane®, Choong Wae Pharm Co., Korea) delivered in oxygen at a flow rate of 4 L/min, without any pre-anesthetics. Anesthesia was maintained for 20-30 minutes to facilitate instrumentation. A 24-G catheter (BD IV Cathete®r, Becton

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Dickinson Korea, Korea) was inserted in central ear vein and a 22-SWG catheter (Green-Cath®, Doo Won Medi-Tech, Korea) was inserted into left dorsal pedal artery. After placing a three-way stop-cock (3-Way Stopcock, Sungwon Medical, Korea), an arterial line (Transpac® IV monitoring kit., Abbott Critical Care Systems, USA) was connected to a pre-calibrated arterial blood pressure transducer (Pulscan-Component®, Scionic Co., USA) for continuous recording of blood pressure and heart rate (HR). The three-way stop-cock was also used for repeated sampling of arterial blood for gases (pH_a, P_aCO_2 and P_aO_2) analysis. Electrodes were attached electrocardiogram (Pulscan-Component®, Scionic Co., USA). Body temperature was monitored using a rectal probe. A pulse oxymetry probe was placed on the tongue to monitor the hemoglobin oxygen saturation (SpO_2).

Anesthesia

All pigs were allowed to recover from anesthesia and then each pig received two different drug treatments: (i) 1 mg/kg xylazine hydrochloride (Rompun®, Bayer Korea, Korea) IM, and 2 mg/kg TZ (Zoletil®, Virbac Labratories, France) IM (group TZX); (ii) 0.5 mg/kg midazolam (Vascam®, Ha Na Pharm Co., Korea) IV, and 2 mg/kg TZ IM (group TZM).

Induction time, recumbency time and standing time were recorded for each pig. Induction time was the time from injection to complete immobilization. Complete immobilization was defined as a lack of response to handling. Anesthesia time was the time interval between complete immobilization and the first attempt made by the animal to lift its head a few centimeters above the ground. Standing time was defined as the time interval between the end of anesthesia until the animal stood without assistance for longer than 10 seconds.

Score of anesthetic effects (sedation, analgesia, muscle relaxation, posture and auditory response) were subjectively evaluated every 15 minutes during anesthesia (Table 1). A score was given to each category. Data are shown as means ± SD.

Cardiopulmonary parameters (heart rate (HR), respiratory rate (RR), rectal temperature (RT), Systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressures) were measured and recorded at the time of 0, 5, 15, 30, 45 and 60 minutes.

Arterial blood samples were collected at the time of 0, 5, 15, 30, 45 and 60 minutes from the arterial catheter. The samples were collected in 1 ml syringes and analyzed (i-STAT®, Portable Clinical Analyzer, Heska Corporation, USA) within immediately.

Table 1. Criteria used to score anesthetic effects of TZX or TZM in pigs

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)

Table 2. Mean (± SD) values (minutes) for induction, recumbency and walking times

Doses	Induction time	recumbency time	Walking time
TZX	4.50 ± 0.50	54.75 ± 4.03	112.00 ± 3.65
TZM	3.57 ± 0.30*	51.75 ± 6.23	106.50 ± 4.43

Data are expressed as mean ± SD (n=4)
*Significantly different ($P < 0.05$) from the TZX

Statistical analysis

The data between group TZX and TZM compared by using Kruskal-Wallis analysis of variance and Friedman test. Results are presented as the mean ± SD. The significance level was set at 0.05. All statistics were performed using a computer statistical package (SPSS for Windows, Release 12.0.1 24 Mar 2004, SPSS Inc., USA).

Result

The anesthesia of all pigs was successful. Both drug combinations provided a smooth induction and good immobilization. Induction, recumbency and standing times are reported in Table 2. Induction time in the TZM group was significantly faster than the TZX group. Recumbency and standing times were not significant between groups.

Degree of sedation score at 45 and 60 minutes was significantly higher in the TZM group than the TZX group. Analgesia and muscle relaxation scores in the TZX group were significantly decreased at 60 minutes after drug administration. Muscle relaxation and posture score were significantly higher at 60 minutes in the TZM group than the TZX group (Table 3).

Heart rate was significantly decreased from baseline at the time of 15, 30, 45 and 60 minutes in the TZX group. In the TZM group, HR significantly decreased at 30 minutes from baseline. There was significant difference between the TZX group and the TZM group at the time of 15, 30, 45 and 60 minutes (Table 4).

Respiratory rate of both groups were changed a little as compared with baseline. There was no significant difference between both groups (Table 4).

In the TZX group, SAP, MAP and DAP at 45 minutes were significantly increased from baseline. MAP of the TZX group was increased at 15 minutes and then, decreased from 15 minutes to the end of anesthesia, but in the TZM group showed opposite aspect (decreased at 15 minutes, and then increased from 15 minutes to end of anesthesia). MAP showed significant difference between the TZX group and the TZM group at 15 and 30 minutes (Table 4).

Rectal temperature of the TZM group was stable during the course of anesthesia. But in the TZX group, rectal temperature was significantly decreased at 15, 30, 45 and 60 minutes. There was no significant difference between both groups (Table 4).

After induction of anesthesia the arterial pH (pH_a) increased in both groups. That was significantly increased at the time of 30, 45 and 60 minutes in the TZX group, at 45 and 60 minutes in the TZM group. No significant difference was observed in both groups (Table 5).

In the TZM group, arterial carbon dioxide (P_aCO_2) significantly increased during induction time (5 min - 51.9 ± 9.8 mmHg) but gradually decreased to 60 minutes (43.2 ± 4.8). In the TZX group, P_aCO_2 decreased during anesthesia. There was no significant difference from baseline also between both groups (Table 5).

The arterial oxygen (P_aO_2) significantly increased at 5 minutes, and then the values gradually increased to 60 min in the TZX group. In the TZM group, P_aO_2 increased to 30 minutes after administration anesthetics. There was significant difference at the time of 30 and 40 minutes, compared with baseline (Table 5). None of the measured conscious variables differed significantly between both groups.

Table 3. Mean values for sedation, analgesia, muscle relaxation, posture and response to noise in pigs after administration of TZX or TZM

Effect	Group	15 min	30 min	45 min	60 min
Sedation	TZX	3.00	2.75 ± 0.50	2.00 ± 0.81	0.25 ± 0.50
	TZM	3.00	3.00	2.75 ± 0.50*	0.50 ± 0.57+
Analgesia	TZX	3.00	2.75 ± 0.50	2.75 ± 0.50	0.75 ± 0.50*
	TZM	3.00	3.00	3.00	1.25 ± 0.50
Muscle relaxation	TZX	3.00	2.75 ± 0.50	2.00 ± 0.81	0.25 ± 0.50*
	TZM	3.00	3.00	2.50 ± 1.00	1.50 ± 0.57+
Posture	TZX	3.00	3.00	2.75 ± 0.50	1.75 ± 0.95
	TZM	3.00	3.00	3.00	2.75 ± 0.50+
Auditory response	TZX	3.00	2.75 ± 0.50	2.25 ± 0.95	1.00 ± 1.41
	TZM	3.00	3.00	2.75 ± 0.50	1.00

Data are expressed as mean ± SD (n = 4)
*Significantly different ($P < 0.05$) from baseline
+Significantly different ($P < 0.05$) from the TZX

Table 4. Mean (± SD) data of heart rate (HR-beats minute^{-1}), respiratory rate (RR-breaths minute^{-1}), systolic arterial pressure (SAP-mmHg), mean arterial pressure (MAP-mmHg), diastolic arterial pressure (DAP-mmHg) and rectal temperature (RT-°C)

	Group	pre	5 min	15min	30min	45min	60min
HR	TZX	127.75 ± 1.97	91.50 ± 1.65*	91.50 ± 1.65*	83.25 ± 2.53*	88.75 ± 1.37*	98.50 ± 4.57*
	TZM	142.75 ± 16.00	142.00 ± 18.73+	131.25 ± 21.80+	119.50 ± 18.39*+	135.00 ± 16.77+	133.75 ± 15.91+
RR	TZX	33.00 ± 3.87	33.50 ± 3.42	36.50 ± 1.93	40.00 ± 1.77	39.50 ± 1.25	42.75 ± 1.75
	TZM	38.25 ± 8.25	36 ± 2.45	35.75 ± 6.01	37.25 ± 7.79	41.75 ± 10.78	41 ± 8.39
SAP	TZX	111.75 ± 2.36	119 ± 19.13	120 ± 13.73	115 ± 7.25	113 ± 6.78*	110.25 ± 4.03
	TZM	103.25 ± 6.39	99.75 ± 11.92	101.75 ± 10.43	104.25 ± 7.80	110 ± 7.70	109.25 ± 10.04
MAP	TZX	79.75 ± 3.86	91.75 ± 21.10	90 ± 14.30	84.5 ± 7.59	84 ± 6.05*	75.75 ± 10.01
	TZM	70.5 ± 2.88	67 ± 6.05+	67.75 ± 7.80+	72.5 ± 6.65+	75.75 ± 5.96	75.75 ± 8.65
DAP	TZX	61.75 ± 8.26	78.75 ± 22.23	73.25 ± 15.81	70.25 ± 10.40	68.25 ± 6.07*	61.75 ± 7.13
	TZM	55.25 ± 4.50	50.75 ± 4.71+	51 ± 7.52+	56.5 ± 8.18	58.75 ± 8.77	58.75 ± 9.87
RT	TZX	36.7 ± 0.74	36.575 ± 0.84	36.4 ± 0.93*	36.05 ± 0.93*	35.85 ± 0.88*	35.75 ± 0.97*
	TZM	36.925 ± 1.22	36.9 ± 1.24	36.825 ± 1.26	36.725 ± 1.39	36.75 ± 1.43	36.725 ± 1.41

Data are expressed as mean ± SD (n = 4)
*Significantly different ($P < 0.05$) from baseline
+Significantly different ($P < 0.05$) from the TZX

Table 5. Mean (± SD) data of arterial pH (pH_a), arterial carbon dioxide (P_aCO_2-mmHg), arterial oxygen (P_aO_2-mmHg), oxygen saturation (SO_2-%)

	Group	pre	5 min	15min	30min	45min	60min
pH_a	TZX	7.368 ± 0.05	7.38375 ± 0.05	7.4075 ± 0.03	7.44025 ± 0.03*	7.44825 ± 0.02*	7.4535 ± 0.02*
	TZM	7.435 ± 0.04	7.43575 ± 0.05	7.4305 ± 0.02	7.4615 ± 0.01	7.47575 ± 0.03*	7.4915 ± 0.04*
P_aCO_2	TZX	49.65 ± 7.22	46.525 ± 5.34	47.7 ± 4.29	45.5 ± 1.28	44.75 ± 1.58	44.85 ± 1.26
	TZM	47.2 ± 10.15	51.9 ± 9.86	50.25 ± 3.99	46.475 ± 2.24	45.1 ± 4.81	43.2 ± 4.87
P_aO_2	TZX	71.25 ± 12.52	76.75 ± 18.17*	73.5 ± 11.93	74 ± 13.19	76.25 ± 11.87	79.75 ± 12.17
	TZM	64.5 ± 1.73	66.25 ± 5.61	70 ± 8.64	79.5 ± 15.92*	78.75 ± 10.53*	73.75 ± 8.65
SO_2	TZX	93.75 ± 1.70	94.25 ± 2.62	94.25 ± 2.06	95 ± 3.16	95.5 ± 1.73	95.5 ± 1.00
	TZM	94 ± 2.70	93.75 ± 2.98	93.75 ± 2.21	95.5 ± 2.08	95 ± 2.44	95.5 ± 1.91

Data are expressed as mean ± SD (n = 4)
*Significantly different ($P < 0.05$) from baseline

Discussion

The benzodiazepines have anxiolytic, amnesic, anticonvulsant, hypnotic and sedative effect, muscle relaxant properties (11), but little or no analgesia. Thus they are generally combined with an anesthetic or strong analgesic to enhance anesthetic action (10). Previous studies found that the sedative effects of midazolam are evident within 3 minutes post-administration and the maximum effect occurred at 15 minutes in pig (1). Midazolam produces a rapid onset and adequate for sedation in pig. But to our knowledge, the combination of midazolam and tiletamine/zolazepam has not been used in pigs for analgesia.

Previous studies have shown that xylazine improves the effects of tiletamine/zolazepam in domestic pigs by increasing muscle relaxation, analgesia and providing a smoother recovery from anesthesia (5,11). The use of tiletamine/zolazepam (4.4-6 mg/kg) and xylazine (1-4 mg/kg) resulted in 30-60 minutes of anesthesia with an induction period of 5 minutes. This combination maintained physiologic variables within acceptable limits while providing satisfactory muscle relaxation and short-term analgesia (5,11).

In the present study, the clinical efficacy of the TZM group was superior to that of the TZX group in pigs. The onset of sedation was significantly longer using the TZX group than the TZM group. The quality of induction and recovery in the TZM group was considered smoother than in the TZM group.

In the anesthetic effect of sedation, analgesia, muscle relaxation and posture scores, the TZM group was much more stable than the in TZX group. When both treatments were compared, we observed a prolonged anesthesia time in the TZM group. All pigs in the TZM treatment remained lateral recumbency and retained analgesia for at least 40 minutes. This is an adequate amount of time to do most procedures that require general anesthesia.

The xylazine can also produce adverse side effects on cardiovascular system, such as arrhythmia, decrease in heart rate

and cardiac output, increasing arterial pressure, decreasing temperature in other species. The activation of α_2 receptor occurs in increasing arterial blood pressure result in vascular smooth muscle contraction and vasoconstriction (9).

In the TZX group, heart rate was significantly decreased below baseline values after the administration of TZX. This finding is consistent with other reports of the effects of xylazine in other species (2,8).

Other studies have shown that intramuscular administration of xylazine is not associated with as dramatic an increase in blood pressure and vascular resistance (3,7). However in our study, blood pressure was significantly higher at the time of 45 minutes.

Generally tiletamine-zolazepam slightly increases heart rate and depresses respiratory system, occasionally causes apnea, but respiratory rate remains unchanged or increased in most species (15). In this study respiratory rate was gradually increased but not significant in both groups.

Arterial pH, P_aCO_2 and P_aO_2 values remain virtually unchanged in other species (3,13). However, in our study arterial pH significantly increased at 30, 45 and 60 minutes in the TZX group. This results shows that hyperventilation occurs to 30 minutes, and then it's same to recovery time in the TZX group.

Midazolam, similar to other benzodiazepines, causes minimal cardiorespiratory changes in pigs (9). In this study, the administration of midazolam and tiletamine-zolazepam combination did not induce significant changes in heart rate (except 30 min), respiratory rates, mean blood pressure and rectal temperature.

The xylazine can also decrease peripheral body temperature result in peripheral vasoconstriction. In this study, the rectal temperature significantly decreased at all anesthesia time in the TZX group. Immobilization trials on domestic pigs using tiletamin-zolazepam and xylazine, previous study noted decreases in body temperatures during pressing (11,14).

In conclusion, the pharmacokinetics of both drug combinations has also not been studied in pigs. When xylazine was used for premedication, it can produce adverse side effects on cardiovascular system.

Midazolam/tiletamine/zolazepam combination has not been used in pigs. But in this study, this drug combination showed rapid induction, adequate immobilization, analgesia, muscle relaxation and minimal cardiopulmonary effects. Thus, this drug combination can be used effectively for the pig anesthesia in experimental research.

This study demonstrated that tiletamine-zolazepam combined with midazolam causes adequate immobilization characterized by rapid induction, adequate analgesia and muscle relaxation with pigs. When the two drug combinations were compared, the TZM group showed minimal cardiorespiratory effects. Also there were fewer side effects in the TZM group rather than the TZX group. Midazolam and tiletamine-zolazepam combination was effective in providing adequate immobilization in pigs.

However in pigs, drug injected by the intramuscular route have become popular for immobilization and for induction of anesthesia. Further investigation is required to evaluate the cardiopulmonary effect of tiletamine-zolazepam combined intramuscular route of midazolam in pigs.

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돼지에서 Zoletil/Midazolam과 Zoletil/Xylazine의 2가지 병용마취에 대한 비교 연구

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요 약 : 이 연구의 목적은 돼지에 있어서 zoletil/midazolam (TZM)과 zoletil/xylazine (TZX)의 2가지 병용마취를 사용하였을 때의 마취효과와 심혈관계 및 호흡에 미치는 영향을 비교하기 위하여 실시하였다. 총 8 마리의 Landrace × Yorkshire 교잡종 돼지 (25.3 ± 3.3 kg)를 사용하였으며, 각 군마다 4 마리씩 2개 군으로 실험을 실시하였다. 첫 번째 군은 xylazine과 tiletamine/zolazepam을 2 mg/kg 용량으로 근육 내 주사하였고(TZX군), 2번째 군은 midazolam 0.5 mg/kg의 용량을 정맥 내 주사 하고 tiletamine/zolazepam 2 mg/kg 용량으로 근육 내 주사하였다(TZM 군). 마취시간에 대한 평가로 induction time, anesthesia time과 standing time을 각 돼지마다 측정하였으며, 마취효과에 대한 평가로 진정, 진통, 근 이완, 자세 그리고 청각반응을 점수화 하여 매 15분마다 측정하였다. 심폐기능에 대한 평가로 심박동수와 동맥혈압, 호흡수, 직장체온을 마취 전, 마취직후, 마취 후 5분, 15분, 30분, 45분 및 60분에 각각 측정하였고, 동맥혈 가스 분석을 동일시간대에 실시하였다. 실험 결과 모든 돼지에서의 마취는 성공적이었다. 2가지 병용마취 모두 부드러운 마취 유도와 적절한 운동억제 효과를 보였으나 마취효과점수에서는 TZM군이 TZX군보다 우수하였으며, TZM군이 TZX군 보다 심폐기능 및 체온에 미치는 영향이 적으며 안정적인 것을 확인하였다. 결론적으로, TZM군은 TZX군에 비하여 더욱 양호한 마취효과를 보였으며, 심폐기능에 미치는 영향은 보다 적게 나타내었다.

주요어 : 마취, midazolam, 돼지, xylazine, zoletil