Anaesthesia and cardiopulmonary effects of tiletamine-zolazepam/xylazine/tramadol and its effects on nitric oxide, plasma endothelin, 6-keto-$PGF_{1\alpha}$ and thromboxanes B_2 in miniature pigs

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Received June 10, 2012
Accepted November 29, 2012

Abstract

This study focused on anaesthesia and cardiopulmonary effects of tiletamine-zolazepam/xylazine/tramadol in miniature pigs and its effects on endothelium-derived vasoactive factors. A total of 14 eight-month-old Chinese experimental miniature pigs were used in this study. Tiletamine-zolazepam (3.5 mg·kg^{-1}), xylazine (1.32 mg·kg^{-1}) and tramadol (1.8 mg·kg^{-1}) were administered i.m.; blood pressure and heart rate were recorded. At the same time, blood was collected through precaval vein, and nitric oxide, endothelin, 6-keto-$PGF_{1\alpha}$ and thromboxanes B_2 were determined by colorimetry and radioimmunoassay. The mean times to dorsal recumbency, duration of immobilization, standing and walking were 2.26 ± 0.72, 87.57 ± 9.61, 25.63 ± 12.55 and 36.70 ± 14.53 min, respectively. Blood pressure was significantly changed at 10 and 80 min ($P < 0.01$), and the heart rate ranged from 89 to 134 bpm without episodes of severe bradycardia or tachycardia. Significantly positive correlation was observed between endothelin, thromboxanes B_2 and blood pressure as well as the heart rate ($P < 0.05$). There was negative correlation between PGI_2 and blood pressure as well as heart rate ($P < 0.05$). The results showed that endothelin, 6-keto-$PGF_{1\alpha}$ and thromboxanes B_2 participated in the changing of cardiopulmonary parameters which were caused by tiletamine-zolazepam-xylazine-tramadol anaesthesia in miniature pigs, and the 6-keto-$PGF_{1\alpha}$, ET and TXB_2 concentrations in plasma participated in the changing of blood pressures during anaesthesia. Therefore, we can recommend tiletamine-zolazepam/xylazine/tramadol for anaesthesia in pigs, and this study also contributes to the evaluation of the effect of endothelium-derived vasoactive factors during anaesthesia.

Swine, cardiopulmonary, endothelium-derived vasoactive factors, correlation

The usefulness of pigs in biomedical research is widely acknowledged. Several characteristics common to pigs and humans indicate that the pig can serve as a good experimental model for human (Sullivan et al. 2001; Raušer et al. 2011). Although juvenile domestic pigs can be used for research, adult miniature pigs are a better approximation of adult human physiology, and they can be difficult to restrain and anaesthetize effectively (Vodicka et al. 2005; Alexa et al. 2011).

A 1:1 proprietary combination of a dissociative anaesthetic, tiletamine, and a benzodiazepine tranquilizer, zolazepam is available commercially (Lin et al. 1993). Tiletamine-zolazepam administered intramuscularly (i.m.) has been reported to induce immobilization but not analgesia or muscle relaxation in pigs (Kim et al. 2007; Raušer et al. 2008). An α_2-adrenoreceptor agonist, xylazine, has been combined with tiletamine-zolazepam to increase its anaesthetic and analgesic effects in pigs (Sweitzer et al. 1997). However, their use is associated with adverse effects including hypertension and unwanted persistence of pharmacological effects (Kreeger 1999). Tramadol is a centrally acting analgesic that is structurally related to codeine and morphine, and exerts its action through interactions with opioid, serotonin and adrenergic receptors (Shilo et al. 2007). It acts as a weak m-opioid agonist coupled with inhibition of synaptic reuptake of serotonin and norepinephrine, achieving spinal modulation of pain and preventing impulses reaching the brain (Monteiro et al. 2009).

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The aim of the present study was to investigate the effect of tiletamine-zolazepam/xylazine/tramadol combination on concentrations of endothelium-derived vasoactive factors and selected cardiopulmonary parameters in Chinese experimental miniature pigs.

Materials and Methods

The procedures and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (20110503) in China. A total of 14 experimental Chinese miniature pigs, seven females and seven males, mean age 8 months old (range 7–10 months) and 27.7 kg (33–22.4 kg) b.w. were used in this study. The pigs were purchased from the China Agricultural University Laboratory Animals Institutes, and all animals had identical housing, feeding and unlimited water intake. During the last 3 months before experiment, they were without any medication or surgery.

All miniature pigs were determined to be in good physical condition based on physical examination and complete blood count. Food, but not water, was withheld for 12 h prior to the beginning of the experiment. Every animal was allowed to acclimate to a room with temperature at 25 ºC for at least 30 min before experiment. Tiletamine/zolazepam-xylazine-tramadol was administered by injection using a hand held syringe with an intended dose of 3.5 $mg \cdot kg^{-1}$ tiletamine-zolazepam (Zoletil® 100, Virbac corporation, Carros, France), 1.32 $mg \cdot kg^{-1}$ xylazine (Rompun, Bayer, Leverkusen, Germany), and 1.8 $mg \cdot kg^{-1}$ tramadol (Tramal® 100; Grunenthal GmbH, Aachen, Germany) into muscles of the caudal thigh region. After immobilization, an ophthalmic ointment was placed in the eyes to prevent corneal drying.

Induction, anesthesia, standing and walking time was evaluated according to Selmi et al. (2003). Baseline cardiopulmonary parameters included the heart rate (HR), noninvasive indirect systolic arterial blood pressure (SAP), diastolic arterial blood pressure (DAP), mean arterial blood pressure (MAP) were measured by noninvasive monitor (Datex-OhmedaS/5, Datex-Ohmeda Division Instrumentarium Corp., Helsinki, Finland) before and 5, 15, 30, 45, 60, 80, 100, 120 min after drug injection. Monitoring of blood pressures was achieved by placing a cuff circumferentially around the left antebrachium of animals, with the cuff width being approximately 40% of the total circumference of the limb. Heart rate was determined by counting heart beats for 1 min using a stethoscope placed at the lower left lateral thoracic wall.

The pig's forelegs were held back and its head pressed down. The area around the thoracic inlet was cleaned with cotton swabs soaked in surgical spirit. 5.0 ml of blood was then collected from the pig by venepuncture of the anterior vena cava as described by Muirhead (1981). Blood samples were collected in vacutainer tubes, after coagulation the blood samples were centrifuged (3,000 *g* / 10 min), serum was extracted and stored at 4 ºC until analysed 5 h after withdrawal. Then serum concentration of nitric oxide was assayed by Nitric Oxide Colorimetric Assay Kit (Abcam Inc., Cambridge, US). Plasma ET, PGI_2 and TXB_2 concentrations were determined using the ET, 6-keto-$PGF_{1\alpha}$ and TXB_2-1,2[125I] assay systems (Amersham International, Amersham, UK). Briefly, after blood samples were collected in tubes, the samples were mixed with anticoagulant by gently rotating the tubes in the palms for about 1 min, immediately centrifuged at 3,000 *g* at 4 °C, and kept in ice packs pending the time of laboratory evaluation.

Cardiopulmonary parameters and levels of endothelium derived vasoactive factors were analyzed by means of ANOVA for repeated measures to evaluate changes within each group, followed by Tukey test to compare values over time. Correlation analysis between cardiopulmonary parameters and levels of endothelium derived vasoactive factors were also analyzed. All statistical analyses were performed using SPSS 13.0 (SPSS- Statistical Product and Service Solutions 13.0, SPSS Incorporation, US). A probability level of 5% ($P < 0.05$) was considered significant. All values are reported as mean ± standard deviation.

Results

The pigs lay down, usually first to sternal recumbency, 1.53 ± 0.85 min after the combination injection and were deeply sedated in lateral recumbency after 2.26 ± 0.72 min. The combination resulted in the anaesthesia lasting about 87.57 ± 9.61 min. Recovery was characterized by slow movements of the eyes, twitching of the ears and attempts of lift the head. Animals were much calmer during recovery and the features such as ataxia and uncontrolled manner were not observed during the period of recovery. In most of the animals, a few attempts were made before they could walk for a short distance.

Cardiopulmonary parameters are shown in Table 1. Administration of tiletamine/zolazepam-xylazine-tramadol to miniature pigs caused changes in HR, SAP, DAP and MAP. The mean HR ranged from 89 to 134 bpm, and without episodes of severe bradycardia or tachycardia. The HR was increased at 5 min after administration, and gradually decreased to baseline values nearly 30 min, then decreased below baseline and significantly decreased

Table 1. Mean (± SD) values for cardiopulmonary parameters evaluated in miniature pigs anaesthetized with tiletamine/zolazepam-xylazine-tramadol.

Time (min)	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (beats/min)
0	126 ± 10	72 ± 6	88 ± 7	110 ± 4
5	131 ± 9	77 ± 7	95 ± 8	134 ± 4*
10	144 ± 10*	82 ± 5*	101 ± 7*	125 ± 3*
30	113 ± 8	73 ± 6	86 ± 7	107 ± 4
45	114 ± 13	72 ± 6	83 ± 8	105 ± 3
60	111 ± 8*	74 ± 8	82 ± 8	93 ± 5*
80	106 ± 10*	69 ± 6	76 ± 8*	89 ± 3*
100	119 ± 16	72 ± 11	85 ± 9	111 ± 2
120	127 ± 10	75 ± 5	92 ± 9	131 ± 4*

SAP – systolic arterial blood pressure, DAP – diastolic arterial blood pressure, MAP – mean arterial blood pressure, HR – heart rate
*significantly ($P < 0.05$) different from values at 0 min within each group

Table 2. Effect of tiletamine/zolazepam-xylazine-tramadol on the serum nitric oxide, plasma endothelin, 6-keto-$PGF_{1\alpha}$ and plasma thromboxanes B_2 concentration in miniature pigs.

Time (min)	NO (μmol·l^{-1})	ET (pg·ml^{-1})	6-keto-PGF1α (pg·ml^{-1})	TXB2 (pg·ml^{-1})
0	36 ± 7	175 ± 17	133 ± 22	191 ± 17
5	27 ± 8	161 ± 16	131 ± 22	179 ± 16
10	23 ± 6*	199 ± 21	121 ± 20	204 ± 16
30	35 ± 7	126 ± 20*	155 ± 33	157 ± 16
45	35 ± 6	132 ± 18	149 ± 30	171 ± 15
60	36 ± 7	145 ± 20	143 ± 27	182 ± 13
80	28 ± 7	112 ± 19*	190 ± 28*	128 ± 13*
100	38 ± 7	154 ± 28	141 ± 31	151 ± 20
120	43 ± 7	131 ± 26	148 ± 27	167 ± 12

NO – nitric oxide, ET – endothelin, TXB_2 – thromboxanes B_2
*significantly ($P < 0.05$) different from values at 0 min within each group.

Table 3. Analysis of correlation between endothelium-derived vasoactive factors and blood pressures, heart rate in miniature pigs anaesthetized with tiletamine/zolazepam-xylazine-tramadol.

	SAP	DAP	MAP	HR
NO	-0.362	-0.488	-0.319	-0.058
ET	0.846*	0.72*	0.762*	0.492
6-keto-$PGF_{1\alpha}$	-0.796*	-0.734*	-0.798*	-0.643
TXB_2	0.742*	0.744*	0.723*	0.472

SAP – systolic arterial blood pressure, DAP – diastolic arterial blood pressure, MAP – mean arterial blood pressure, HR – heart rate, NO – nitric oxide, ET – endothelin, TXB_2 – thromboxanes B_2
*significantly ($P < 0.05$) different from correlation coefficient

at 80 min. This study showed that the haemodynamics changed significantly ($P < 0.05$) in miniature pigs after administration of drugs, but these changes were within biologically acceptable limits.

Concentrations of endothelium-derived vasoactive factors are shown in Table 2. The values of NO showed a significant ($P < 0.05$) decrease at 10 min, returning to baseline at 120 min ($P > 0.05$). There were some changes in ET, 6-keto-$PGF_{1\alpha}$ and TXB_2, and significant differences occurred at 80 min ($P < 0.05$). Similarly, these variables did not display any obvious changes at 120 min ($P > 0.05$).

Correlation analysis is shown in Table 3. The correlation between endothelium-derived vasoactive factors and the heart rate were -0.058, 0.492, -0.643, and 0.472, respectively, and there were no differences between them ($P > 0.05$). Non-significant correlation was found between NO values and blood pressure ($P > 0.05$), but significant correlation was found between blood pressure and other endothelium-derived vasoactive factors (ET, 6-keto-$PGF_{1\alpha}$, TXB_2) ($P < 0.05$).

Discussion

Alpha-two adrenoreceptor agonists such as xylazine play a role in the modulation of blood pressure, degree of alertness, gastrointestinal electrolyte absorption, papillary diameter, and blood glucose concentration (James et al. 1999). Some studies have demonstrated that xylazine can improve the effects of tiletamine/zolazepam in swine by increasing muscle relaxation, analgesia and providing smoother recovery from anaesthesia (Sweitzer et al. 1997). But it is frequently associated with bradycardia, which could be due to decreased sympathetic activity and/or increased vagal tone (Selmi et al. 2003). Tramadol has proven effective in both experimental and clinical pain without causing serious cardiovascular or respiratory adverse effects (Witte et al. 2001).

In miniature pigs anaesthetized with tiletamine/zolazepam-xylazine-tramadol combination, the heart rate was changed immediately after drug administration. Because tramadol produced less pronounced cardiovascular depression effects (Witte et al. 2001), the results in our study suggested that the positive chronotropic effects of tiletamine/zolazepam probably counterbalance temporarily and partially the bradycardic effect of xylazine (Selmi et al. 2003; Kim et al. 2007). In our study, blood pressure changed after anaesthetic drug combination administration, but the values were well maintained in the animals.

Endothelium-derived vasoactive factors play an important role in the regulation of the vascular system. These factors include nitric oxide, prostacyclin, and an unidentified hyperpolarizing factor which causes relaxation; those causing contraction include angiotensin II, endothelin, oxygen-derived free radicals, prostacyclin I_2, and thromboxane B_2 (Shepherd and Katusić 1991). In our study, we measured NO, PGI_2, ET and TXA_2 concentrations, but PGI_2 and TXA_2 are unstable, so we used 6-keto-$PGF_{1\alpha}$ and TXB_2 concentrations in place of PGI_2 and TXA_2 concentrations. Nitric oxide and 6-keto-$PGF_{1\alpha}$ are known to be endothelium-derived relaxing factors, and ET and TXB_2 are known to be endothelium-derived contracting factors. Although blood pressures and HR had some changes after tiletamine/zolazepam-xylazine-tramadol combination administration, these values returned to baseline at the end of monitoring ($P > 0.05$). The results showed that ET and TXB_2 had positive correlation with blood pressure and 6-keto-$PGF_{1\alpha}$ had negative correlation with blood pressure. These results also indicated that 6-keto-$PGF_{1\alpha}$, ET and TXB_2 had participated in changing the blood pressure in miniature pigs anaesthetized with the tiletamine/zolazepam-xylazine-tramadol combination.

We can conclude that tiletamine/zolazepam-xylazine-tramadol combination produced satisfactory immobilization and anaesthesia in experimental Chinese miniature pigs, and

the 6-keto-$PGF_{1\alpha}$, ET and TXB_2 concentrations in plasma participated in changing the blood pressure during anaesthesia. The results of this study support the use of tiletamine/zolazepam-xylazine-tramadol as an immobilization technique for miniature pigs.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant No. 30671552 and 30972227).

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