

Effects of the tiletamine/zolazepam-xylazine-tramadol combination on plasma oxidative status and haematological indicators in miniature pigs

Sheng Jiang¹, Hong-Gang Fan¹, De-Zhang Lu^{1,2}, Jin-Long Hou¹, Xu-Dong Song¹, Yue Wang¹, Hong-Bin Wang¹

¹Northeast Agricultural University, College of Veterinary Medicine, Harbin, China

²Northwest A&F University, College of Veterinary Medicine, Yangling, Shanxi, China

Received September 16, 2013

Accepted January 23, 2014

Abstract

Oxidative stress in the body occurs when the production of free radicals overwhelms the antioxidant defence systems. The aim of this study was to examine the effects of a tiletamine/zolazepam-xylazine-tramadol combination on the antioxidant status, lipoperoxidase and blood cell indicators in eight miniature pigs. Baseline measures were recorded prior to anaesthesia, then the animals were anaesthetized with the combination of tiletamine/zolazepam (3.5 mg/kg), xylazine (1.32 mg/kg), and tramadol (1.8 mg/kg). Blood samples were collected from the anterior vena cava at 15, 30, 60, 90, 120 min, 24 h, and 72 h after anaesthesia. Plasma malondialdehyde, superoxide dismutase, catalase, and glutathione peroxidase concentrations were measured by colorimetry, and red blood cell counts, white blood cell counts, haemoglobin and packed cell volume were determined using an automated cell counter. The results showed that the concentration of malondialdehyde increased significantly at 30 and 60 min after the injection ($P < 0.05$), whereas glutathione peroxidase and catalase activity increased slightly ($P > 0.05$) then returned to baseline values after 90 min ($P > 0.05$). Superoxide dismutase activity increased significantly ($P < 0.05$) at 30 and 60 min, and then gradually decreased to baseline values after 90 min. Changes in red blood cell counts, haemoglobin and packed cell volume were not significant, while white blood cell count decreased significantly ($P < 0.05$) at 30 and 60 min. Our study is the first to demonstrate that tiletamine/zolazepam-xylazine-tramadol provide antioxidant effects, which may be proposed for alleviating the stress of examination and research at veterinary clinics or long-distance transportation.

Anaesthesia, lipoperoxidase, blood cell, oxidant/antioxidant, swine

Oxyradicals are produced as by-products both during normal metabolism, i.e., mitochondrial respiration, and under oxidative stress. Under normal circumstances, the generated reactive oxygen species (ROS) are detoxified via enzymatic and non-enzymatic antioxidant defences present in the body when the generated ROS and antioxidants are within the physiological equilibrium range. Enzymatic systems involved include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and non-enzymatic antioxidants include urate, cysteine, vitamin E, vitamin C, and β -carotene (Aydilek 2007; Fidan et al. 2009). Oxidative stress in the body occurs when the production of free radicals overwhelms the antioxidant defence systems, resulting in damage of DNA and certain membrane lipids (Halliwell 1994). Thus, antioxidants help to counterbalance such reactions and play a vital role in cellular protection (Sies 1997). The pro-oxidation effects of some general anaesthetics have been described (Khinev and Dafinova 1993), and various anaesthetics possessing different physicochemical properties have been reported to affect the lipid peroxidation process directly or indirectly, leading to the formation of malondialdehyde (MDA) in the body or even to tissue damage.

Tiletamine/zolazepam is an injectable drug combination used widely by veterinarians in mammalian species, alone or in combination with other anaesthetic agents (Williams et al. 2002; Ko et al. 2007; Re et al. 2011; Lu et al. 2013). Xylazine is an α_2 -adrenergic receptor

Address for correspondence:

Hong-Bin Wang, PhD
College of Veterinary Medicine
Northeast Agricultural University
Harbin 150030, China

E-mail: hbwang1940@163.com
<http://actavet.vfu.cz/>

agonist sedative with analgesic properties that has a significant effect on depression of the heart rate and the respiratory system (Derossi et al. 2003). Tramadol is an effective centrally acting analgesic (Lee et al. 1993) that additionally acts as a monoamine reuptake inhibitor (Desmeules et al. 1996). Tramadol is sometimes referred to as atypical opioid and its analgesic activity may be mediated by both opioid and non-opioid (i.e. norepinephrine and serotonin reuptake inhibition) mechanisms (Lewis and Han 1997; Liu et al. 1999; Nossaman et al. 2010). The combination of tiletamine/zolazepam-xylazine-tramadol (TZXT) has been reported to produce safe and adequate anaesthesia in an earlier study on miniature pigs (Lu et al. 2010). However, to our knowledge, no study has examined the effect of the TZXT combination on plasma oxidant/antioxidant indicators in miniature pigs.

In the present study, we aimed to investigate the effect of TZXT on the activities of GSH-Px, CAT and SOD, serum MDA and several haematological indicators before, during, and after anaesthesia of miniature pigs.

Materials and Methods

After approval by the Northeast Agricultural University Committee on Animal Care and Use, eight (3 female and 5 male) healthy 8-month-old (age range 7–10 months) Chinese experimental miniature pigs (27.7 kg, range 33–22.4 kg) in good physical condition were selected for the procedure. They were housed individually and fed dry commercial dog food (Pedigree®, Mars Petcare China, Beijing, China) twice daily and water *ad libitum*. A routine physical examination (i.e., complete blood count, biochemical profile and electrocardiogram) was carried out two days before the study, and in all animals, all variables were within the physiological limits. All animals appeared healthy and exhibited no clinical signs of disease.

Following a 12 h starvation period, the pigs were anaesthetised with 3.5 mg/kg tiletamine/zolazepam (Zoletil® 100; Virbac Corporation, Carros, France), 1.32 mg/kg xylazine (Rompun®; Bayer, Leverkusen, Germany) and 1.8 mg/kg tramadol hydrochloride (Tramal® 100; Grunenthal GmbH, Aachen, Germany). All drugs were administered intramuscularly. The pigs were placed in lateral recumbence, breathing air spontaneously without intubation. Anaesthesia was produced within 3 min (2–8 min on average) and lasted approximately 80 min (mean 87.57 ± 9.61 min).

Prior to anaesthesia, 4 ml blood samples were collected by venipuncture from the anterior vena cava into EDTA tubes (0 h; control), then at 15, 30, 60, 90, 120 min, 24 h and 72 h following anaesthesia. All of the 4 ml of blood collected, 2 ml was retained for haematological analyses, and the remaining 2 ml samples were immediately centrifuged at $1500 \times g$ for 10 min and the resulting plasma samples stored at -80°C until required for measurement of other indicators. Red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV) and haemoglobin concentration (Hb) were determined using an automated cell counter (Coulter MD18, Beckman Coulter, Brea, CA, USA). The concentrations of MDA were measured spectrophotometrically with MDA Assay Kit A003 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) by the thiobarbituric acid (TBA) method (Asakawa and Matsushita 1980). Activity of SOD was determined using a SOD Assay Kit A001-1 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) according to the method cited by Oyanagui (1984). This method is based on the measurement of the inhibition of SOD-induced formation of nitrite from hydroxylamine by spectrophotometry. Activity of CAT was determined with CAT Assay Kit A007 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) according to the colorimetric method (Beers and Sizer 1952). Activity of GSH-Px was determined with a GSH-Px Assay Kit A005 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The GSH-Px has the ability to decompose hydrogen peroxide and other organic hydroperoxides. The reaction uses glutathione to complete the reaction using hydrogen peroxide, as the substrate. Consumption of nicotinamide adenine dinucleotide phosphate (NADPH) was used to determine the GSH-Px activity.

All data are expressed as means \pm standard deviation (SD). Statistical analysis of data within groups was performed with one-way analyses of variance for repeated measures followed by the least significant difference test. The $P < 0.05$ was considered significant. All statistical analyses were carried out using SPSS v13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

All pigs recovered from anaesthesia without complications and no excitement was observed during anaesthesia. The values of CAT and GSH-Px activity, MDA concentration and haematological indicators are shown in Table 1. After anaesthetic injection, MDA concentration increased, and its values at 30 and 60 min were significantly higher compared

Table 1. Changes in haematological and oxidant/antioxidant indicators before and after anaesthesia in miniature pigs.

Indicator	Time							
	0 min	15 min	30 min	60 min	90 min	120 min	24 h	72h
GSH-Px ($\mu\text{mol/l}$)	1120.72 \pm 158.71	1172.94 \pm 146.68	1205.07 \pm 216.81	1214.07 \pm 157.60	1188.90 \pm 286.44	1142.31 \pm 175.01	1162.62 \pm 146.87	1117.95 \pm 163.67
SOD (U/ml)	59.36 \pm 4.85	60.11 \pm 5.97	63.15 \pm 4.53*	65.54 \pm 2.62*	60.47 \pm 3.29	60.63 \pm 4.46	60.29 \pm 4.24	61.49 \pm 5.57
MDA (nmol/ml)	2.51 \pm 0.13	2.80 \pm 0.14	3.39 \pm 0.30*	3.68 \pm 0.37*	3.02 \pm 0.64	2.98 \pm 0.43	3.06 \pm 0.55	2.48 \pm 0.50
CAT (U/ml)	2.64 \pm 1.21	2.84 \pm 0.40	3.01 \pm 0.68	2.93 \pm 0.56	3.01 \pm 0.98	2.83 \pm 0.71	2.73 \pm 0.52	2.70 \pm 0.82
RBC $\times 10^{12}/\text{l}$	15.86 \pm 4.58	13.39 \pm 4.57	12.11 \pm 2.10	12.99 \pm 3.09	13.42 \pm 3.13	14.12 \pm 3.12	15.91 \pm 5.54	14.09 \pm 3.16
WBC $\times 10^9/\text{l}$	10.17 \pm 1.24	9.45 \pm 1.63	8.85 \pm 1.53*	8.07 \pm 0.98*	9.35 \pm 1.48	9.29 \pm 1.53	10.32 \pm 1.65	9.57 \pm 1.81
PCV (%)	55.39 \pm 11.57	50.97 \pm 10.16	49.53 \pm 7.47	46.22 \pm 8.25	48.46 \pm 10.85	47.12 \pm 10.12	51.72 \pm 12.46	54.86 \pm 9.58
Hb (g/l)	147.32 \pm 36.23	142.42 \pm 31.55	140.31 \pm 34.27	142.57 \pm 33.28	145.78 \pm 34.17	147.29 \pm 35.53	149.32 \pm 31.68	144.58 \pm 48.23

GSH-Px – glutathione peroxidase, SOD – superoxide dismutase, MDA – malondialdehyde, CAT – catalase, RBC – red blood cell, WBC – white blood cell, PCV – packed cell volume, Hb – haemoglobin. Data are expressed as mean \pm SD, *significantly different ($P < 0.05$) values compared with 0 min within each group.

to baseline values ($P < 0.05$). Activity of SOD increased significantly at 30 and 60 min ($P < 0.05$), then gradually decreased to baseline value at 90 min. There was a non-significant increase in the GSH-Px and CAT activity after TZXT administration, and its values returned to baseline values after 90 min ($P > 0.05$). The RBC counts, PCV and Hb decreased, but no significant changes were observed ($P > 0.05$). The WBC counts showed a statistically significant decrease at 30 and 60 min ($P < 0.05$), returning to baseline values at 90 min ($P > 0.05$).

Discussion

The main enzymes that control the biological effects of reactive oxygen species are SOD, CAT and GSH-Px. The SOD catalyses the dismutation of superoxide anions into H_2O_2 , GSH-Px oxidizes reduced glutathione, inactivates H_2O_2 and reduces organic peroxides to their alcohols (Michels et al. 1994), and CAT catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen (Isaksson et al. 2009). Tramadol can inhibit the re-uptake of norepinephrine between the synapsis (Raffa et al. 1992), so the concentrations of norepinephrine may increase. Norepinephrine can exert neuroprotective effects by acting as an extracellular antioxidant (Rommelfanger and Weinshenker 2007) and intraperitoneal injections of norepinephrine can result in an increase of SOD and CAT activities (Danielisová et al. 2006). The increase in SOD may be due to the non-opioid mechanisms of Tramadol.

A previous study in humans reported no significant change in MDA concentrations before and after anaesthesia (Khinev et al. 1995). However, other studies examining

the use of tiletamine-zolazepam (Ceylan et al. 2007) or its combination with xylazine in sheep (Aydilek 2007) and gazelles (Yaralioglu-Gurgoze et al. 2005) demonstrated an increase in MDA concentrations, similar to the results of our study. Based on the fact that MDA is one of the end products of lipid peroxidation, and is both an indicator and effector of oxidative stress (Sivaci et al. 2006) our study may support the fact that oxygen free radicals are released upon administration of TZXT. Our data demonstrated that animals injected with TZXT had increased plasma SOD, GSH-Px and CAT activity. These increases act to maintain the oxidant/antioxidant balance, indicating that TZXT is capable of increasing the antioxidant capacity in pigs.

In a previous study in sheep, tiletamine-zolazepam alone caused a significant decrease in Hb and PCV (Isaksson et al. 2009). However, the combination of tiletamine-zolazepam-xylazine had no effect on certain haematological indicators in dogs and gazelles (Yaralioglu-Gurgoze et al. 2005). In the present study, RBC, PCV and Hb decreased gradually and returned to baseline levels after injection of TZXT. Pooling of the circulating blood cells in the spleen or other reservoirs secondary to decreased sympathetic activity may explain the decrease in Hb, PCV and WBC (Singh et al. 2007). The significant decrease in WBC may be related to capture of the animals prior to injection. The decrease in PCV and Hb during the period of anaesthesia or sedation may also be a result of the shifting of fluid from the extravascular compartment to the intravascular compartment (Wagner et al. 1991) in order to maintain normal cardiac output.

In conclusion, our results demonstrate that administration of TZXT has available antioxidant effects in miniature pigs. These findings may be beneficial to animals in which free radicals play a role in oxidative stress, such as during long-distance transportation. Additionally, the findings may support the use of TZXT for chemical immobilization of miniature pigs and may be proposed for alleviating the stress from veterinary clinics examination and research.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant no. 31272617), Science and Technology Research Project (General Program) of Heilongjiang Provincial Department of Education (Grant No. 12521003) and Doctoral Scientific Research Foundation of Northeast Agricultural University (2009RC60).

References

- Asakawa T, Matsushita S 1980: Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. *Lipids* **15**: 137-140
- Aydilek N 2007: Comparison between xylazine-tiletamine-zolazepam and fentanyl-tiletamine-zolazepam anaesthetic combinations on plasma oxidative status in sheep. *Acta Vet Brno* **76**: 573-578
- Beers RF, Sizer IW 1952: A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Bio Chem* **195**: 133-140
- Ceylan C, Aydılek N, Ipek H 2007: Effects of tiletamine-zolazepam anaesthesia on plasma antioxidative status and some haematological parameters in sheep. *Acta Vet Hung* **55**: 191-197
- Danielisová V, Némethová M, Gottlieb M, Burda J 2006: The changes in endogenous antioxidant enzyme activity after postconditioning. *Cell Mol Neurobiol* **26**: 1179-1189
- Derossi R, Junqueira AL, Beretta MP 2003: Analgesic and systemic effects of ketamine, xylazine, and lidocaine after subarachnoid administration in goats. *Am J Vet Res* **64**: 51-56
- Desmeules JA, Piguet V, Collart L, Dayer P 1996: Contribution of monoaminergic modulation to the analgesic effect of tramadol. *Br J Clin Pharmacol* **41**: 7-12
- Fidan AF, Kucukkurt I, Eryavuz A, Cigerci IH, Yardimci M, Ozdemir A 2009: Effects of shearing procedures on oxidant-antioxidant status in Chios sheep. *Revue Med Vet* **160**: 349-355
- Halliwel B 1994: Free radicals and antioxidants: A personal view. *Nutr Rev* **52**: 253-265
- Isaksson C, Sturve J, Almroth BC, Andersson S 2009: The impact of urban environment on oxidative damage (TBARS) and antioxidant systems in lungs and liver of great tits, *Parus major*. *Environ Res* **109**: 46-50
- Khinev S, Dafinova K 1993: The effect of general anaesthesia and its components on free radical processes. *Khirurgiia Sofia* **46**: 49-52
- Khinev S, Dafinova K, Tenchova V, Bakalova R 1995: The lipid peroxidation level and antioxidant status of the plasma in patients operated under propofol (diprivan) anesthesia. *Khirurgiia Sofia* **48**: 23-25
- Ko JC, Payton M, Weil AB, Kitao T, Haydon T 2007: Comparison of anesthetic and cardiorespiratory effects of tiletamine-zolazepam-butorphanol and tiletamine-zolazepam- butorphanol-medetomidine in dogs. *Vet Ther* **8**: 113-126
- Lee CR, McTavish D, Sorkin EM 1993: Tramadol: A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute and chronic pain states. *Drugs* **46**: 313-340
- Lewis KS, Han NH 1997: Tramadol: a new centrally acting analgesic. *Am J Health-Syst Ph* **54**: 643-652
- Liu ZM, Zhou WH, Lian Z, Mu Y, Ren ZH, Cao JQ, Cai ZJ 1999: Drug dependence and abuse potential of tramadol. *Acta Pharmacol Sin* **20**: 52-54
- Lu DZ, Fan HG, Jiang S, Tan LJ, Yu SM, Zhang LS, Ma K, Wang HB 2013: Anaesthesia and cardiopulmonary effects of tiletamine-zolazepam/xylazine/tramadol and its effects on nitric oxide, plasma endothelin, 6-keto-PGF_{1α} and thromboxanes B₂ in miniature pigs. *Acta Vet Brno* **82**: 103-107

- Lu DZ, Fan HG, Wang HB, Hu K, Zhang JT, Yu SM 2010: Effect of the addition of tramadol to a combination of tiletamine-zolazepam and xylazine for anaesthesia of miniature pigs. *Vet Rec* **167**: 489-492
- Michels C, Raes M, Toussaint O, Remacle J 1994: Importance of Se-glutathione peroxidase, catalase, and Cu/Zn SOD for cell survival against oxidative stress. *Free Radic Biol Med* **17**: 235-248
- Nossaman VE, Ramadhyani U, Kadowitz PJ, Nossaman BD 2010: Advances in perioperative pain management: use of medications with dual analgesic mechanisms, tramadol & tapentadol. *Anesthesiol Clin* **28**: 647-666
- Oyanagui Y 1984: Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal Biochem* **142**: 290-296
- Raffa RB, Fridrichs E, Reimann W, Shank RP, Codd EE, Vaught JL 1992: Opioid and nonopioid components in dependently contributes to the mechanism of action of tramadol, an 'atypical' opioid analgesic. *J Pharmacol Exp Ther* **260**: 275-285
- Re M, Blanco-Murcia FJ, Gómez de Segura IA 2011: Chemical restraint and anaesthetic effects of a tiletamine-zolazepam/ketamine/detomidine combination in cattle. *Vet J* **190**: 66-70
- Rommelfanger KS, Weinshenker D 2007: Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochem Pharmacol* **74**: 177-190
- Sies H 1997: Oxidative stress: oxidants and antioxidants. *Exp Physiol* **82**: 291-295
- Singh K, Kinjavdekar P, Amarpal, Aithal HP, Gopinathan A, Singh GR, Pawde AM, Pratap K 2007: Effects of epidural ketamine-xylazine combination on the clinicophysiological and haematobiochemical parameters of uraemic and healthy goats. *Vet Res Commun* **31**: 133-142
- Sivaci R, Kahraman A, Serteser M, Sahin DA, Dilek ON 2006: Cytotoxic effects of volatile anesthetics with free radicals undergoing laparoscopic surgery. *Clin Biochem* **39**: 293-298
- Wagner AE, Muir WW 3rd, Hinchcliff KW 1991: Cardiovascular effects of xylazine and detomidine in horses. *Am J Vet Res* **52**: 651-657
- Williams LS, Levy JK, Robertson SA, Cistola AM, Centonze LA 2002: Use of the anesthetic combination of tiletamine, zolazepam, ketamine, and xylazine for neutering feral cats. *J Am Vet Med A* **220**: 1491-1495
- Yaralioglu-Gurgoze S, Sindak N, Sahin T, Cen O 2005: Levels of glutathione peroxidase, lipoperoxidase and some biochemical and haematological parameters in gazelles anaesthetized with a tiletamin-zolazepam-xylazine combination. *Vet J* **169**: 126-128