Veterinarski Glasnik 2021, 75 (1), 76-82
UDC: 636.98.09:616-089.5
https://doi.org/10.2298/VETGL181208005V

Full Research Article

PLASMA GLUTATHIONE PEROXIDASE (GPX 3) ACTIVITY IN THE FRESHWATER TURTLE *TRACHEMYS SCRIPTA ELEGANS* AFTER ISOFLURANE INHALATION ANESTHESIA

VALČIĆ Olivera[1], MILANOVIĆ Svetlana[1], DOŠENOVIĆ Milan[2], ÖZVEGY Jòzsef[3], RADAKOVIĆ Milena[4], VEJNOVIĆ Branislav[5], VUČIĆEVIĆ Miloš[2]

[1]University of Belgrade, Faculty of Veterinary Medicine, Department of Physiology and Biochemistry; [2]University of Belgrade, Faculty of Veterinary Medicine, Department of Equine, Small Animal, Poultry and Wild Animal Diseases; [3]Belgrade Zoo, Belgrade, Serbia; [4]University of Belgrade, Faculty of Veterinary Medicine, Department of Pathophysiology; [5]University of Belgrade, Faculty of Veterinary Medicine, Department of Economics and Statistics

Received 08 December 2018; Accepted 31 January 2019
Published online: 25 February 2019

Abstract

Introduction. Glutathione peroxidases are selenoenzymes which have a crucial role in the protection of animals against oxidative stress.

Materials and Methods. From September 2017 to April 2018, a group of eight red-eared sliders were admitted at the Clinic for Small Animals, Faculty of Veterinary Medicine, University of Belgrade for elective diagnostic celioscopy. The turtles were of unknown age, weight from 1.20 kg to 1.86 kg. The anesthesia protocol involved using ketamine and medetomidine, both at a low dosage (10 mg kg^{-1} and 0.1 mg kg^{-1}, respectively) as induction, after which anesthesia was maintained using isoflurane at 3% (vapor setting) in 100% oxygen (0.5 L min^{-1}). Medetomidine was reversed with atipamezole (0.2 mg kg^{-1}), given intramuscularly. The elective celioscopy was performed according to standard protocols. One day prior to anesthesia, heparinized blood samples were taken using the subcarapacial venous plexus for venipuncture. The second sampling took place three hours after the anesthetics were administered.

Results and Conclusions. GPx3 activity in the blood plasmas (n=8) was measured by the coupled test as described by Günzler et al. (1974). Data were tested for normality by the Shapiro-Wilk normality test and the groups were compared using a paired t-test.

Blood plasma GPx3 activity was significantly higher (p=0.009) after a three-hour recovery period from inhalation anesthesia performed for elective diagnostic celioscopy, than before anesthesia. The measured post-anesthesia GPx3 activities were, on average,

*Corresponding author – e-mail: olja@vet.bg.ac.rs

80% higher than the measurements prior to anesthesia. It can be concluded that the statistically significant increase in the activity of plasma GPx3 from 91.02±36.05 μKat L^{-1} prior to anesthesia to 160.21±58.94 μKat L^{-1} three hours after anesthesia is due to the change in oxygen saturation. This is increased to 100% during the procedure, thus exposing the turtles to conditions of high oxygen saturation.

Key Words: glutathione peroxidase, red-eared slider, inhalation anesthesia

INTRODUCTION

Red-eared slider turtle is the common name of the species *Trachemys scripta elegans* of the order Testudine, family Emydidae. Their preferred habitats are still or quiet waters with muddy bottoms and a lush aquatic vegetation. Basking is important for thermoregulation as the optimal body temperature of 28°C is maintained by getting energy from the sun. If the temperature falls below the critical level of 10°C, they will go underwater into a hibernation-like state, i.e. brumation, when they absorb oxygen only through their skin. The sex of the red-eared hatchling is determined by the environmental temperature. Thus, if it is above 29°C, females will develop, and if it is below 29°C, males (Gibbons, 1990).

Due to their specific habitat and lifestyle, red-eared sliders spend long periods underwater and experience wide variations in oxygen availability. Hence they have evolved as a species to be anoxia-tolerant. Their body has to deal with rapid changes in tissue oxygenation with little or no accumulation of damaging products.

Captive breeding of turtles has become more common in the recent years because of the increased popularity of reptiles as pets, as well as the need for captive breeding for conservation purposes (Innis, 2010). The red-eared slider is also an example of a common pet that is widely used in laboratory research, and consequently, there is an increasing need to anaesthetize turtles for clinical examination, diagnostics, and surgical procedures (Greer et al., 2001). Reptiles have a tolerance for anoxia and ischemia that allows them to endure stress such as breath-hold diving and environmental oxygen lack as part of their lifestyles (Lutz and Storey, 1997). Surgical and diagnostic procedures requiring inhalation anesthesia of the red-eared slider are challenging from the aspect of oxidative stress that is additionally imposed on their metabolism.

Antioxidative defense is based on non-enzymatic effectors such as vitamins E and C, ß-carotene, uric acid and enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). GPx is the general name for a family of isoenzymes that catalyze the reduction of hydrogen peroxide or organic hydroperoxides to less toxic compounds such as water or corresponding alcohols. The GPx isoenzymes differ not only in the tissue in which they occur, but also according to their distinct subcellular locations, as GPx1 is an intracellular enzyme, while GPx3 is mainly secreted into the extracellular fluid, i.e. blood plasma, by the epithelial cells of the proximal nephron tubule and by the parietal cells of the Bowman's capsule (Yoshimura et al., 1991). GGPx5 and GPx6 have been identified only in mammals (Margis et al., 2008).

The aim of this study was to investigate the effects of inhalation isoflurane anesthesia applied prior to diagnostic celioscopy on the activity of GPx3 before and after anesthesia of the red-eared slider.

MATERIALS AND METHODS

From September 2017 to April 2018, a group of eight red-eared sliders was admitted at the Clinic for Small Animals, Faculty of Veterinary Medicine, University of Belgrade, for elective diagnostic celioscopy in order to assess their reproductive health status. The turtles were of unknown age, weight from 1.20 kg to 1.86 kg.

Prior to examination, the animals were kept under controlled environment conditions in a water tank at 22°C water temperature and 21-25°C room temperature. The acclimatization time was 14 days.

The anesthesia protocol involved using ketamine (Ketamidor 10% Richter Pharma, Austria) and medetomidine (Domitor, Orion Pharma, Finland) both in a low dosage (10 mg kg^{-1}, 0.1 mg kg^{-1} respectively) as induction, after which anesthesia was maintained using isoflurane (Isoflurane, CP-Pharma, Austria) at 3% (vapor setting) in 100% oxygen (0.5 L min^{-1}), delivered through a modified urinary catheter of diameter 2.6 mm, inserted into the trachea. All turtles breathed atmospheric air during sedation and were allowed to breathe spontaneously during anesthesia.

Medetomidine was reversed with atipamezole (Antisedan, Orion, Finland), 0.2 mg kg^{-1}, given intramuscularly (IM) immediately after elective diagnostic celioscopy was completed (which was approximately one hour after application of anesthetics).

All animals were given postoperatively 20 ml of saline SC, meloxicam (Movalis, Boehringer Ingelheim, Germany) 0.5 mg kg^{-1} IM, and enrofloxacin (Baytril, Bayer Animal Health, Germany) 10 mg kg^{-1} IM, in order to prevent possible infections.

The diagnostic elective celioscopy was performed according to standard surgical protocols in an equal fashion for all the turtles in the study, thus avoiding individual differences in the treatment.

One day prior to anesthesia, heparinized blood samples were taken using the subcarapacial venous plexus for venipuncture. The second sampling took place three hours after the anesthetics were administered.

The GPx3 activity (n=8) in the blood plasmas were measured by the coupled test as described by Günzler et al. (1974). Briefly, the GPx3 present in the plasma (20 µl) reduces tert-butyl hydroperoxide (TBH). Glutathione (GSH: 200µl, final concentration 6 mM), as the donor of hydrogen, becomes oxidized. In the second, coupled reaction, the oxidized GSH (GSSG) is reduced back to GSH by NADPH (200 µl; 0.30 mM) and glutathione reductase (GR, 50µl, 0,375 IU ml^{-1}) Two minutes after TBH addition (500 µl, 1.575 mM), absorbance readings at 366 nm were taken at 60 sec intervals during 3 min on a Cecil Ce2021spectrophotometer with a Peltier thermostat. The optimal pH was provided by 500 µl phosphate buffer.

Statistical analysis

Data normality was accepted using the Shapiro-Wilk normality test ($p>0.05$), and groups were compared using a paired t-test. Significant difference was estimated at $p<0.05$ and $p<0.01$ significance levels. Data are presented as mean±SEM. Statistical analysis was performed with GraphPad Prism version 6 (GraphPad, San Diego, CA, USA).

RESULTS

Red-eared slider blood plasma GPx3 activity was significantly higher ($p= 0.009$) after a three hour recovery period from inhalation anesthesia performed for elective diagnostic celioscopy, compared to the basal values, before anesthesia. The measured post-anesthesia values were, on average, higher by almost 80% compared to the measurements prior to anesthesia (Table 1).

Table 1. Plasma GPx3 activities (μKat L^{-1}) before and three hours after isoflurane inhalation anesthesia

Group	X±SD	CV%	T-test
Before anesthesia	91.02±36.05	39.60	0.009**
After anesthesia	160.21±58.94	36.79	

**$p<0.01$

DISCUSSION

Among vertebrates, anoxia tolerance has developed in a number of freshwater turtles that dive and often hibernate underwater. The *Trachemys* genera (such as the red-eared slider) can survive for months submerged in cold, deoxygenated water (Ultsch, 1989). Under such circumstances, the animals produce ATP under anaerobic conditions, and freshwater turtles also have improved buffering capacity of their cells to deal with high lactic acidosis. It is logical to assume that animals that in nature experience wide variations in oxygen availability express a range of biochemical adaptations. Such adaptations do not only deal with the lack of oxygen, but have to allow the animals' biochemistry to react to the sudden reintroduction of oxygen into their systems when air breathing is resumed. If reactive oxygen species (ROS) and oxidative stress over-generation are a problem during reperfusion after ischemia in mammals, it is logical to assume it can also be a problem during exposition to high oxygen pressure during anesthesia.

Inhalation isoflurane anesthesia certainly is a state in which the available oxygen concentration greatly varies during the time of induction/anesthesia/awakening. It is common practice to administer inhalation anesthesia with an oxygen saturation of 100%, thus exposing turtles to unnatural, hyperoxic conditions. Such sudden

changes in the available oxygen concentration can be compared to variations due to anoxia in the case of diving. Willmore et al. (2001) have studied the effects of anoxia exposure and aerobic recovery on metabolic enzyme activities in the red-eared slider. Those authors reached the conclusion that the enzymatic make-up of turtle organs is well designed to meet the metabolic demands of anoxic excursions. During aerobic recovery, the activity of most enzymes rebounded, although the activities of some other enzymes were not at all affected by anoxia or recovery. Storey (1996) reported that concurrent to enzymatic adaptations to changing partial pressures of available oxygen, levels of reduced GSH and GSSG in the liver and muscle of the red-eared slider are significantly higher than in frogs and snakes. This adds to the hypothesis that anoxia-tolerant reptiles such as the red-eared slider have developed mechanisms of protection against reperfusion injury. In turtles, total GSH content decreased significantly during anoxia exposure in the liver, heart, white muscle and kidney by 49, 67, 54 and 59%, respectively Storey (1996).

It can be postulated that a facultative anaerobe such as the red-eared slider deals with the unavoidable burst of ROS during reperfusion by maintaining high activities of antioxidative enzymes and a large GSH pool. However, the need to administer inhalation anesthesia to reptiles, such as freshwater turtles, has raised the question of the effects of high oxygen saturation on antioxidative enzymes. The antioxidative enzymes involved in the protection against ROS and oxidative stress are: SOD, CAT and Se-dependent GPx. These observations are supported by the findings published by Joanisse and Storey (1996), and Hermes-Lima and Storey (1993, 1995), who reported that tissues from the anoxia-tolerant turtle *T. scripta elegans* clearly have very high activities of these enzymes compared with those of garter snakes and leopard frogs, species that seldom encounter rapid changes in oxygen availability.

A study (Willmore and Storey, 1997) on the effects of anoxic submergence and subsequent aerobic recovery of the red-eared slider showed minimal changes in the concentration of lipid peroxidation products during anoxia or recovery, suggesting that natural anoxic-aerobic transitions occur without the damage that is seen during ischemia-reperfusion in mammals. Anoxia exposure led to decreased enzyme activities, consistent with a reduced potential for oxidative damage during anoxia: SOD decreased in the liver by 30%, CAT decreased in the heart by 31%, CAT and total GPx decreased in the kidney (68 and 41%, respectively). The results show that the adaptation for natural anoxia tolerance in turtles includes well-developed antioxidant defenses that minimize or prevent damage by ROS during reoxygenation after anoxic submergence.

CONCLUSION

It can be concluded that the significant increase in the activity of plasma GPx3 at three hours after anesthesia compared with pre-anesthesia levels is due to the change in oxygen concentration. This is increased to 100% during the procedure, thus exposing the turtles to conditions of extreme and unnatural high oxygen concentrations.

There is a shortage of published data on the effects of inhalation anesthesia administered concurrently with 100% oxygen at a rate of 0.5 L min^{-1} on *T. scripta elegans* enzyme activities, metabolism and oxidative stress, and we consider these to be the first results on the subject, thus supporting the need for further studies.

REFERENCES

Gibbons J. W. 1990. Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Institute Press.

Greer L. L., Jenne K. J., Diggs H. 2001. Medetomidine-ketamine anesthesia in red-eared slider turtles (*Trachemys scripta elegans*). Contemporary Topics, 40(3):8-11.

Günzler W. A., Kremers H., Flohé L. 1974. An improved coupled test procedure for glutathione peroxidase (E.C. 1.11.1.9) in blood. Clinical Chemistry and Laboratory Medicine (CCLM), 12(10):444–448. https://doi.org/10.1515/cclm.1974.12.10.444

Hermes-Lima M., Storey K. B. 1993. Antioxidant defenses in the tolerance of freezing and anoxia by garter snakes. American Journal of Physiology, 265(3):646-652. https://doi.org/10.1152/ajpregu.1993.265.3.R646

Hermes-Lima M., Storey K. B. 1995. Antioxidant defenses and metabolic depression in a pulmonate land snail. American Journal of Physiology, 268(6):1386-1393. https://doi.org/10.1152/ajpregu.1995.268.6.R1386

Innis C. J. 2010. Endoscopy and Endosurgery of the Chelonian Reproductive Tract, Veterinary Clinics: Exotic Animal Practice, 13(2):243–254, doi:10.1016/j.cvex.2010.01.005

Joanisse D. R., Storey K. B. 1996. Oxidative damage and antioxidants in *Rana sylvatica*, the freeze tolerant wood frog. American Journal of Physiology, 271(3):545-553. https://doi.org/10.1152/ajpregu.1996.271.3.R545

Lutz P. L., Storey K. B. 1997. Adaptations to variations of oxygen tensions by vertebrates and invertebrates. In *Handbook of Comparative Physiology*, Boca Raton: CRC Press

Margis R., Dunanad C., Teixeira F. K., Margis-Pinheiro M. 2008. Glutathione peroxidase family – an evolutionary overview. FEBS Journal, 275(15):3959-3970. https://doi.org/10.1111/j.1742-4658.2008.06542.x

Storey K. B. 1996. Oxidative stress: animal adaptations in nature. Brazilian Journal of Medical and Biological Research, 29(12):1715-1733.

Ultsch G. R. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles and snakes. Biological Reviews, 64(4):435-515. https://doi.org/10.1111/j.1469-185X.1989.tb00683.x

Yoshimura S., Wantanabe K., Suemizu H., Onozawa T., Mizoguchi J., Tsuda K., Hatta H., Moriuchi T. 1991. Tissue specific expression of the plasma glutathione peroxidase gene in rat kidney. Journal of Biochemistry, 109(6):918-923. https://doi.org/10.1093/oxfordjournals.jbchem.a123480

Willmore G. W., Cowan K. J., Storey K. B. 2001. Effects of anoxia exposure and aerobic recovery on metabolic enzyme activities in the freshwater turtle *Trachemys scripta elegans*. Canadian Journal of Zoology, 79(10):1822-1828. https://doi.org/10.1139/z01-149

Willmore W. G., Storey K. B. 1997. Antioxidant systems and anoxia tolerance in a freshwater turtle Trachemys scripta elegans. Molecular and Cellular Biochemistry, 170(1-2):177-185. https://doi.org/10.1023/A:1006817806010

AKTIVNOST GLUTATION PEROKSIDAZE (GPX 3) U PLAZMI SEMIAKVATIČNIH KORNJAČA *TRACHEMYS SCRIPTA ELEGANS* NAKON INHALACIONE ANESTEZIJE IZOFLURANOM

VALČIĆ Olivera, MILANOVIĆ Svetlana, DOŠENOVIĆ Milan, OZVEGI Jožef, RADAKOVIĆ Milena, VEJNOVIĆ Branislav, VUČIĆEVIĆ Miloš

Kratak sadržaj

Uvod. Glutation peroksidaze su selenoenzimi koji igraju ključnu ulogu u zaštiti životinja od oksidativnog stresa.

Materijal i metode. Od septembra 2017. do aprila 2018. godine, grupa od osam crvenouhih kornjača je primljena na Kliniku za male životinje, Fakulteta Veterinarske Medicine, Univerziteta u Beogradu zbog izvođenja elektivne dijagnostičke celioskopije. Kornjače su bile nepoznate starosti, mase između 1,20 kg i 1,86 kg. Anastetički protokol je uključivao primenu ketamina (10 mg kg^{-1}) i medetomidina (0.1 mg kg^{-1}) za indukciju, oba u niskim dozama, nakon čega je anestezija održavana primenom izoflurana u koncentraciji od 3% (podešen na isparavanje) u 100% kiseoniku (0.5 L min^{-1}). Dejstvo medetomidina je neutralizovano atipamezolom (0.2 mg kg^{-1}), aplikovanim intramuskularno. Elektivna celioskopija je izvedena u skladu sa standardnim protokolima. Heparinizovani uzorci krvi su uzeti jedan dan pre uvođenja u anesteziju venepunkcijom iz subkarapacijalnog venskog pleksusa. Drugo uzorkovanje krvi je uzvršeno tri sata nakon administracije anestetika.

Rezultati i zaključak. GPx3 aktivnost u krvnoj plazmi (n=8) je izmerena primenom kuplovane reakcije, kao što su opisali Günzler i sar. (1974). Normalna distribucija podataka je testirana pomoću Shapiro - Wilk testa normalnosti ($p>0,05$), a grupe su poređene koristeći t test za zavisne uzorke.

Aktivnost GPx3 u krvnoj plazmi je bila značajno viša ($p=0.009$) u uzorcima uzetim nakon trosatnog perioda oporavka od inhalacione anestezije aplikovane radi izvođenja elektivne celioskopije, nego u uzorcima uzetim pre aplikovanja anestezije. Vrednosti GPx3 izmerene nakon anestezije bile su u proseku 80% više od vrednosti pre anestezije. Može se zaključiti da je statistički značajan porast u aktivnosti GPx3 u plazmi od 91.02 ± 36.05 μKat L^{-1}, pre anestezije do 160.21 ± 58.94 μKat L^{-1}, tri sata nakon anestezije uzrokovan promenom u saturaciji kiseonika. Tokom intervencije saturacija kiseonika se povećava i do 100% čime se kornjače uvode u uslove visoke saturacije kiseonikom.

Ključne reči: glutation peroksidaza, crvenouha kornjača, inhalaciona anestezija