Waltham International Symposium:
Pet Nutrition Coming of Age

Feline Antioxidant Enzyme Activity: The Effect of Sample Storage on Stability[1,2]

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EXPANDED ABSTRACT

KEY WORDS: • *feline* • *superoxide dismutase* • *glutathione peroxidase* • *enzyme stability*

Free radicals and other reactive oxygen species (ROS[4]) formed in vivo have been implicated in the pathology of many clinical and age-related diseases such as cancer, cardiovascular disease and cataracts. The primary cellular defense against the deleterious effects of free radicals and ROS is provided by the antioxidant defense system. In this system, the cupro-enzyme superoxide dismutase (SOD) is the catalyst for the dismutation of the highly reactive superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2). Glutathione peroxidase (GSH-Px) works in conjunction with SOD to break down the ROS of H_2O_2 and other peroxides into water and oxygen (**Fig. 1**).

Biological samples used for the determination of SOD and GSH-Px activity are usually stored at −80°C. However, the effect on enzyme activity of varying storage conditions is not well documented. The purpose of this study was to assess the effect of storage at −80°C on the activity of erythrocyte SOD and whole blood GSH-Px in feline samples.

MATERIALS AND METHODS

Commercially available colorimetric assay kits (1,2) based on an indirect xanthine–xanthine oxidase method for the measurement of SOD as described by Halliwell and Gutteridge (3) and the method of Paglia and Valentine (4) for measurement of GSH-Px were validated to determine erythrocyte SOD and whole blood GSH-Px in the domestic cat (*Felis domesticus*). The intra- and interassay coefficients of variation for both test kits demonstrated acceptable levels of variation of <10%. Small volume blood samples for SOD measurement were collected into potassium EDTA tubes and aliquotted into 0.5-mL samples. The erythrocytes were isolated by centrifugation at 3000 rpm for 10 min at 4°C. Erythrocytes were then washed four times by repeated suspension in 0.9% saline solution and centrifugation as stated. Erythrocyte pellets were analyzed fresh or stored until required. The effect of freezing at −80°C on the level of SOD activity was evaluated in 10 feline samples when fresh and at 1, 3, 7, 14, 21

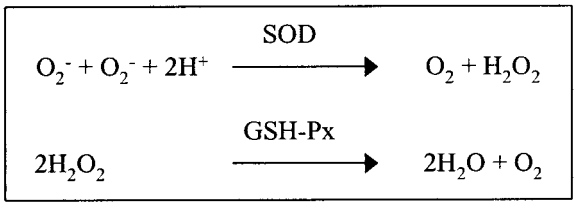

FIGURE 1 The action of antioxidant enzymes on ROS.

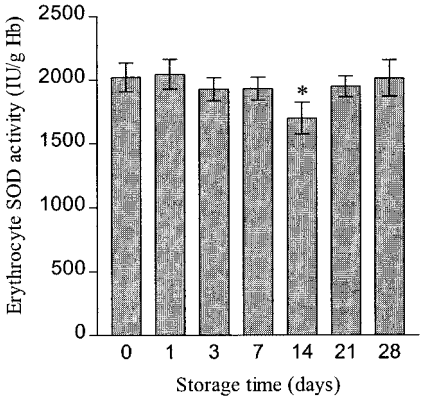

FIGURE 2 The effect of storage at −80°C on feline erythrocyte SOD activity ($P < 0.05$; data are shown as means ± SE, $n = 10$).

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[4] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; GSH-Px, glutathione peroxidase; LSD, least-significant difference; ROS, reactive oxygen species; SOD, superoxide dismutase.

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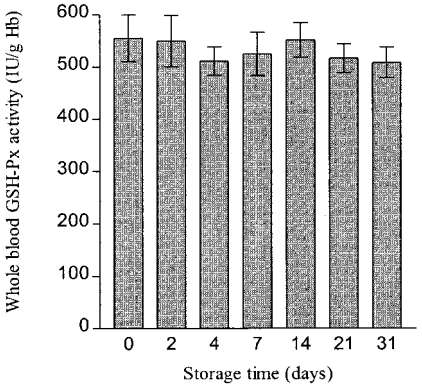

FIGURE 3 The effect of storage at −80°C on feline whole blood GSH-Px activity (data are shown as means ± SE, $n = 8$).

and 28 d after storage. Small-volume whole blood samples were collected into lithium heparin tubes and aliquotted into sample volumes to determine GSH-Px activity. Activity was measured in eight feline samples when fresh and at 2, 4, 7, 14, 21, 31 and 63 d after storage. Enzyme activity for both SOD and GSH-Px was determined per unit of hemoglobin. Fisher's least-significant difference (LSD) test was used to analyze all data.

RESULTS AND DISCUSSION

Erythrocyte SOD activity was significantly reduced after 14 d of storage at −80°C ($P < 0.05$) (**Fig. 2**). However, no significant differences were observed between fresh SOD activity and the activity at the other time points measured. This suggests that the result from the 14-d time point is probably erroneous because of assay variation and can be discounted. There was no significant difference observed between fresh whole blood GSH-Px activity and the activity after 31 d of storage at −80°C (**Fig. 3**).

These results indicate that storage of samples at −80°C is suitable for subsequent determination of SOD and GSH-Px for up to 1 mo. Further research will be required to assess the long-term stability of these antioxidant enzymes.

LITERATURE CITED

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