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Original Research

Investigation of the Antioxidant Status in Canine Tumours

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Abstract

Reactive oxygen species were thought to take part in oncogenesis and cellular differentiation. The study was conducted to know about the ROS levels in dog tumors. The concentration of TBARS, SOD, GSH and GST were estimated in tumor tissues from 68 dogs after the surgical excision. Tumor tissues showed higher concentration TBARS, SOD, GSH and GST as compared to control tissues. Relative concentrations of ROS were also higher in the malignant tumor compared benign tumors. Tissue concentrations of ROS can be used as a marker to detect the presence and grading of tumors.

Keywords: Dogs, GSH, GST, Reactive Oxygen Species, SOD, TBARS, Tumors

Introduction

Reactive oxygen species (ROS), including free radicals and non-radical species, are generated as byproducts of normal cell metabolism. They can be generated within the cell and can act within the cell or can be released to the extracellular space (Frei, 1994). Oxidative damage to cellular macromolecules (lipids, proteins, or nucleic acids) by an excess of reactive oxygen species (oxidative stress) is believed to be involved in the pathogenesis of cancer (Halliwell and Gutteridge, 1999). ROS can seriously alter the structure of molecules such as proteins, lipids and deoxyribonucleic acid (DNA) and these alterations may result in cell degeneration and death causing aging (Hermans *et al.,* 2007), and play a significant role in the pathogenesis of many diseases, such as cardiovascular diseases, neuropathies, inflammatory diseases, acquired immune deficiency syndrome (AIDS), diabetes mellitus, renal diseases and mammalian cancers (Macotpet *et al.,* 2013). In dogs, oxidative stress has been associated with carcinogenesis (Winter *et al.,* 2009). The ROS target primarily the polyunsaturated fatty acids in cell membranes and cause lipid peroxidation. The products of lipid peroxidation may induce DNA damage and consequently mutagenesis and carcinogenesis (Rajneesh *et al.,* 2008). The biomarkers of lipid peroxidation that can be measured in both blood and tissues is thiobarbituric acid-reactive substances (TBARS) and serum malondialdehyde (Szczubial et al., 2008). Antioxidative defense mechanisms using endogenous enzymes and diet-derived antioxidants moderate the effects of ROS. Oxidant –antioxidant profile studies were carried in human cancers (Gönenç *et al.,* 2006 and Rajneesh *et al.,* 2008) and few similar studies performed in dog tumors (Kumaraguruparan *et al.,* 2006 and Karayannopoulou *et al.,* 2013).

The aim of present study was to estimate TBARS, superoxide dismutase (SOD), reduced glutathione (GSH) and Glutamyl S transferase (GST). The TBARS levels in the tissues were analysed to determine the extent of lipid peroxidation and the activity of SOD and GSH were assessed as they form the major components of antioxidant defense. GST was assessed as it is an important enzyme of xenobiotics metabolism.

Materials and Methods

To study the Oxidant-Antioxidant Profile of canine neoplasms, samples were collected from the surgical cases coming to the Teaching Veterinary Clinical Complex, college hospital and different hospitals in and around Hyderabad. The collected samples were tumour tissues and adjacent normal tissues. From each dog, 1gm of tumour tissue was collected, washed with normal saline, and immediately frozen (–70°C) for later determination of oxidative stress. After confirmative diagnosis of tumours, enzyme analysis was carried for tumour tissues and normal tissues served as control.

The tissue TBARS, SOD, GST and GSH concentration was assayed by a spectrophotometric method (Hitachi U-2000 spectrophotometer, Tokyo, Japan) as the assays results in colored chromogen. The tissues were homogenized (10%) in 1.15% KCl and analyzed for thiobarbituric acid reactive substances (TBARS) using Ohkawa *et al.* (1979) method. Similarly, 10% homogenate of tissues was prepared in 0.2 M sodium phosphate buffer pH 8.0 for the estimation of glutathione (Moron *et al.,* 1979) and SOD levels (Madesh *et al.,* 1998). The data was subjected to statistical software (SPSS) using two tailed t-test (Snedecor and Cochran, 1994).

Results and Discussion

A total of 68 samples were collected, of them 31 (45.59%) were benign and 37 (54.41%) were malignant tumours. Tumours were classified as 20 mammary tumours (31.74%), 25 skin tumours (36.76%), 12 mesenchymal tumours (17.64%) and 11 round cell tumours (16.17%). The highest risk of development of various tumours was found in the age group of 7-9 years, followed by 4-6 years, above 9 years and below 3 years and the incidence was 29 (42.65%), 20(29.41%),19 (27.94%) and 1 (1.47%), respectively. The TBARS, SOD, GSH and GST levels in the tumour tissue were raised significantly compared to the normal tissues. The mean values were given in the Table 1.

The generation of ROS is thought to be linked to the carcinogenesis at different levels. Oxidative damage to the DNA has been demonstrated in vitro and in vivo leading to the DNA damage, double strand breaks, rearrangement which result in point mutation, deletion or gene amplification as a first step in carcinogenesis. ROS also activate various cancer signaling pathways and transcription factors in tumour cells regulating proliferation, metastasis and angiogenesis, thus contributing towards cancer progression. Further, ROS are capable of deactivating, detoxifying the enzymes responsible for scavenging of potent carcinogens (Behrend *et al.,* 2003). Since oxidative stress triggers

various antioxidant mechanisms in the body, biomarkers such as lipid peroxidation products and endogenous enzymes with antioxidant properties have been identified and used to assess oxidative stress in mammals.

Table 1: Oxidant and antioxidant concentrations in different canine tumours

TBARS levels (n moles/gm)		
Type of tumour	**Normal tissue**	**Tumour tissue**
Mammary tumours	122.07 ± 11.02	298.06 ± 6.42*
Skin tumours	110.56 ± 8.56	276.10 ± 4.26*
Mesenchymal tumours	43.24 ± 3.26	79.21 ± 2.25*
Round cell tumours	99.85 ± 3.49	192.31 ± 10.35*
SOD (units/mg of protein)		
Mammary tumours	15.23±3.43	28.43±4.23*
Skin tumours	10.68±2.39	18.02±2.47*
Mesenchymal tumours	6.10±2.04	10.09±1.64*
Round cell tumours	8.29±1.32	12.87±2.56*
GSH levels (mg/gm of protein)		
Mammary tumours	16.53±1.63	26.09±2.41*
Skin tumours	10.51±1.12	18.96±3.45*
Mesenchymal tumours	6.76±1.81	9.47±2.89*
Round cell tumours	8.46±1.58	12.75±1.05*
GST levels (µ moles/min/gm)		
Mammary tumours	1.98±0.43	4.02±0.82*
Skin tumours	1.72±0.87	3.81±0.52*
Mesenchymal tumours	0.98±0.23	1.21±0.77*
Round cell tumours	1.32±0.34	1.52±0.64*

*Values were expressed as Mean±S.E.*indicates statistical significance at the 0.05 level of significance*

The best way to assess the relationship between oxidative stress and ROS is to measure ROS directly. However, ROS are difficult to measure with standard biochemical techniques due to their high reactivity and short half-life. Therefore, surrogate markers are required and MDA/TBARS is commonly used as a biomarker of oxidative stress for various pathological conditions and diseases, including cancers (Winter *et al.*, 2009 and Chole *et al.*, 2010).

In the present study, it was clear that TBARS levels were significantly higher in cancer tissues compared to normal tissues. Increased lipid peroxidation due to reactive oxygen species (ROS)-induced oxidative stress has been demonstrated in the tissue of dogs tumors. Few studies on dogs with mammary gland tumors demonstrated that TBARS levels were significantly higher in the tumor tissue than in the normal tissue (Kumaraguruparan *et al.*, 2006, Karayannopoulou *et al.*, 2013 and Jayasri *et al.*, 2013). It is theorized that most cancers in older dogs is due to an increase in free radicals with age and free radical invasion of DNA over a long period. This results in DNA damage, mutations, and possibly carcinogenesis and ultimately the loss of cell function and cell death (Langseth, 1999). Increased blood levels of lipid peroxidation products are usually metabolized and detoxified rapidly after the oxidative challenge, and thus their tissue measurement has been advisable than blood (Gonenc *et al.*, 2001). Hence in this study, tissues were used.

A significant role is played by antioxidant enzymes in protecting the cells from oxidative damages. Superoxide dismutase's (SODs), glutathione peroxidases and catalases are the main antioxidant enzymes present in cell and Superoxide dismutase (SOD) specifically catalyse superoxide radical (O_2-) conversion to H_2 O_2 and O_2. SODs thus protect oxygen-metabolizing cells from the detrimental effects of superoxide free radicals and are therefore considered as key enzymes for controlling oxidative stress (Fridovich, 1995). SODs are present in mammalian cells in three isoforms namely SOD_1, SOD_2 and SOD_3. SOD_2 down regulation was initially associated with tumor initiation and thus it was proposed as a tumor suppressor gene (Oberley, 2005). Recent studies have reported that

SOD_2 might favor tumor progression and dissemination, increased SOD2 expression in some cancers like lung, colorectal and prostrate and other carcinomas have been found to be associated with metastasis and poor disease outcomes (Miar *et al.,* 2015). Thus, it has been proposed that higher SOD_2 levels correlate with the aggressiveness, increased metastatic potential (Malafa *et al.,* 2000) and poor prognosis (Miranda *et al.,* 2000). In present study also there is significant increase in level of SODs were observed. Few reports on dog mammary tumours there is increased SOD level (Kumaraguruparan *et al.,* 2006 and Jayasri *et al.,* 2013). Therefore, recently it has been looked upon as a potential marker during progression from tumor growth to metastasis.

In tumours tissues levels of GSH and GST increased significantly ($P<0.05$) in comparison to the normal tissues. GST was involved in the detoxification of electrophilic toxins which were the end products of carcinogens. The levels of GST were increased in human tumour tissues (Saydam *et al.,* 1997). GSH is a non-protein thiol which was essential for intracellular redox balance in conjuction with GPx and GST. Elevation of GSH levels is an early proliferative response that has been found to change redox status of cell resulting in activation of genes essential for G1 to S transition. Enhanced GSH levels were attributed to detoxification of reactive intermediates generated during biotransformation of xenobiotics. The findings were in accordance with others (Ghalia and Fouad, 2000 and Mohan and Nagini, 2003). There is increased level of GST and GSH were also observed in canine mammary tumors (Kumaraguruparan *et al.,* 2006 and Jayasri *et al.,* 2013). Increased GSH expression is reported in canine mammary tumors without ulceration, not metastatic tumors, and low mortality (Leonel *et al.,* 2014). Hence, elevated GSH in canine mammary tissues may be believed to contribute both to antioxidant defense and cell proliferation.

Conclusion

In conclusion, that oxidative stress is associated with many types of cancers in dogs, and ROS parameters viz TBARS, SOD, GST and GSH levels were reported significantly higher in tumour tissues compared to normal tissues. Tissue concentrations of ROS can be used as a marker to detect the presence though not specific and grade of tumors, when tissue samples collected from tumour mass.

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Conflict of Interests

There is no conflict of interest.

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References

1. Behrend, L., Henderson, G., & Zwacka, R. M. (2003). Reactive oxygen species in oncogenic transformation. *Biochem Society Trans*, 31, 1441-1444.
2. Chole, R. H., Patil, R. N., Basak, A., Palandurkar, K., & Bhowate, R. (2010). Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. *Journal of cancer research and therapeutics*, *6*(4), 487.
3. Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: mechanisms of action. *The American journal of medicine*, *97*(3), S5-S13.
4. Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annual review of biochemistry*, *64*(1), 97-112.
5. Ghalia, A. H. A., & Fouad, I. M. (2000). Glutathione and its metabolizing enzymes in patients with different benign and malignant diseases. *Clinical Biochemistry*, *33*(8), 657-662.
6. Gönenç, A., Erten, D., Aslan, S., Akıncı, M., Şimşek, B., & Torun, M. (2006). Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. *Cell biology international*, *30*(4), 376-380.

7. Gönenç, A., Özkan, Y., Torun, M., & Şimşek, B. (2001). Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. *Journal of Clinical Pharmacy and Therapeutics*, *26*(2), 141-144.
8. Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford University Press, USA. 246–320.
9. Hermans, N., Cos, P., Maes, L., De Bruyne, T., Vanden Berghe, D., J Vlietinck, A., & Pieters, L. (2007). Challenges and pitfalls in antioxidant research. *Current Medicinal Chemistry*, *14*(4), 417-430.
10. Jayasri, K., Padmaja, K., & Saibaba, M. (2016). Altered oxidative stress and carbohydrate metabolism in canine mammary tumors. *Veterinary World*, *9*(12), 1489.
11. Karayannopoulou, M., Fytianou, A., Assaloumidis, N., Psalla, D., Savvas, I., & Kaldrymidou, E. (2013). Lipid peroxidation in neoplastic tissue of dogs with mammary cancer fed with different kinds of diet. *Turkish Journal of Veterinary and Animal Sciences*, *37*(4), 449-453.
12. Kumaraguruparan, R., Balachandran, C., Manohar, B. M., & Nagini, S. (2005). Altered oxidant–antioxidant profile in canine mammary tumours. *Veterinary Research Communications*, *29*(4), 287-296.
13. Langseth, L. (1999). Oxidants, Antioxidants, and Disease Prevention. *Washington DC:ILSI Press*;102-108.
14. Leonel, C., Gelaleti, G. B., Jardim, B. V., Moschetta, M. G., Regiani, V. R., Oliveira, J. G., & Zuccari, D. A. (2014). Expression of glutathione, glutathione peroxidase and glutathione S-transferase pi in canine mammary tumors. *BMC Veterinary Research*, *10*(1), 49.
15. Macotpet, A., Suksawat, F., Sukon, P., Pimpakdee, K., Pattarapanwichien, E., Tangrassameeprasert, R., & Boonsiri, P. (2013). Oxidative stress in cancer-bearing dogs assessed by measuring serum malondialdehyde. *BMC Veterinary Research*, *9*(1), 101.
16. Madesh, M., & Balasubramanian, K. A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry & Biophysics*, *35*(3), 184-188.
17. Malafa, M., Margenthaler, J., Webb, B., Neitzel, L., & Christophersen, M. (2000). MnSOD expression is increased in metastatic gastric cancer. *Journal of Surgical Research*, *88*(2), 130-134.
18. Miar, A., Hevia, D., Muñoz-Cimadevilla, H., Astudillo, A., Velasco, J., Sainz, R. M., & Mayo, J. C. (2015). Manganese superoxide dismutase (SOD2/MnSOD)/catalase and SOD2/GPx1 ratios as biomarkers for tumor progression and metastasis in prostate, colon, and lung cancer. *Free Radical Biology and Medicine*, *85*, 45-55.
19. Miranda, A., Janssen, L., Bosman, C. B., van Duijn, W., Oostendorp-van de Ruit, M. M., Kubben, F. J. & van de Velde, C. J. (2000). Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. *Clinical Cancer Research*, *6*(8), 3183-3192.
20. Mohan, K. C., & Nagini, S. (2003). Dose-response effects of tomato lycopene on lipid peroxidation and enzymic antioxidants in the hamster buccal pouch carcinogenesis model. *Nutrition Research*, *23*(10), 1403-1416.
21. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta (BBA)-general subjects*, *582*(1), 67-78.
22. Oberley, L. W. (2005). Mechanism of the tumor suppressive effect of MnSOD overexpression. *Biomedicine & Pharmacotherapy*, *59*(4), 143-148.
23. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358.
24. Rajneesh, C. P., Manimaran, A., Sasikala, K. R., & Adaikappan, P. (2008). Lipid peroxidation and antioxidant status in patients with breast cancer. *Singapore Medical Journal*, *49*(8), 640.
25. Saydam, N., Kirib, A., Demir, O., Hazan, E., Ote, O., Saydam, O., & Guner, G. (1997). Determination of reduced glutathione, glutathione peroxidase and glutathione S transferase levels in human lung cancer. *Cancer Letters*, 199, 13-19.
26. Snedecor, G. W., & Cochran. WG. (1994) Statistical Methods. 8th ed. Ames: *Iowa State University Press. Ames, Iowa*, 62-82.
27. Szczubiał, M., Kankofer, M., Łopuszyński, W., Dąbrowski, R., & Lipko, J. (2004). Oxidative stress parameters in bitches with mammary gland tumours. *Journal of Veterinary Medicine Series A*, *51*(7-8), 336-340.
28. Winter, J. L., Barber, L. G., Freeman, L., Griessmayr, P. C., Milbury, P. E., & Blumberg, J. B. (2009). Antioxidant status and biomarkers of oxidative stress in dogs with lymphoma. *Journal of Veterinary Internal Medicine*, *23*(2), 311-316.

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