ALTERED ANTIOXIDANT PROFILE AND CARBOHYDRATE METABOLISM IN CANINE MAMMARY TUMORS

K. Jayasri, K. Padmaja, P. Eswara Prasad, M. Saibaba

Department of Veterinary Biochemistry, C.V.Sc, S.V.V.U, Tirupati.
INTRODUCTION

- Mammary tumors are the most common and prevalent type of neoplasms in canine species.

- ROS can cause DNA damage, activate pro-carcinogens, and alter the cellular antioxidant defense system (Ray and Husain, 1990).

- Altered antioxidant enzymes during carcinogenesis (Yi sun 1990, Kumaramurugappan et al., 2013)
INTRODUCTION

- high rate of proliferation
- High ATP
- Increased biosynthesis of macromolecules
- Tightened maintenance of appropriate cellular redox status.

- High rate of glucose metabolism is metabolic hallmark of rapidly dividing cells (De Berardinis et al., 2008).
INTRODUCTION

- High rate of cellular proliferation in hypoxic tumor environment hinders the aerobic pathways inside the cell.

- Cell metabolism is shifted towards glycolysis.

- Lack of data about metabolic alterations in canine mammary tumors.
## MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
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<tbody>
<tr>
<td>TBARS</td>
<td>Okhawa et al., 1975</td>
</tr>
<tr>
<td>GSH</td>
<td>Ellman, 1959</td>
</tr>
<tr>
<td>GPx</td>
<td>Rotruck et al., 1973</td>
</tr>
<tr>
<td>GST</td>
<td>Habig, 1974</td>
</tr>
<tr>
<td>Catalase</td>
<td>Misra &amp; Fridovich, 1972</td>
</tr>
<tr>
<td>Protein</td>
<td>Lowry et al., 1951</td>
</tr>
<tr>
<td>Hexose</td>
<td>Niebes, 1972</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>Brandstrup et al., 1957</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Koida and Oda, 1959</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
<td>Gancedo and Gancedo, 1971</td>
</tr>
<tr>
<td>Glucose-6-Phosphate Dehydrogenase</td>
<td>Ellis and Kirkman, 1961</td>
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**RESULTS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal mammary gland</th>
<th>Mammary tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (μmoles/g tissue)</td>
<td>13.73±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.17±3.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutathione (mg /100g tissue)</td>
<td>32.0 ±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134 ±9.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catalase (U/mg protein)</td>
<td>0.71 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 ±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>7.07 ±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.27 ±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GST (U/mg protein)</td>
<td>10.58 ±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.64 ±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean±SE
Means are significantly different (p <0.001)
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<tr>
<th>Parameter</th>
<th>Normal mammary gland</th>
<th>Mammary tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose (mg/g tissue)</td>
<td>184 ±15.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>506.67 ±28.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexokinase (µ moles of glucose phosphorylated/min/mg protein)</td>
<td>0.41 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 ±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphatase (µ moles of inorganic ph released/min/mg protein)</td>
<td>0.067 ±0.0041&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.045 ±0.0013&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase (µ moles of inorganic ph released/min/mg protein)</td>
<td>7.04 ±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.96 ±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (mIU/mg protein)</td>
<td>0.245 ±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.054 ±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are Mean±SE
 Means are significantly different (p <0.001)
DISCUSSION

- Increase in lipid peroxidation may be due to the overproduction of oxygen free radicals.

- Damage to biomolecules such as DNA, lipids, proteins and carbohydrates (Datta et al., 2000).

- MDA reacts with DNA to form adducts and induces mutations in proto-oncogenes and tumor suppressor genes which lead to transformation (Burcham, 1998).

- Further, rapidly dividing cells causes excess production of oxygen free radicals due to increased metabolism (Kumaramurugappan et al., 2005).
GLUTATHIONE

- Glutathione, a thiol containing compound.

- Elevation of GSH levels is an early proliferative response (Poot et al., 1995).

- Maintain the reduced status of thioredoxin which is essential for DNA synthesis (Lu, 1999).

- The reduced activities of GPx, GST and catalase in cancer tissues are responsible for increase in TBARS formation.
  This may be due to the excessive utilization of enzymes in conjugation process.
Normal cells proliferation resulting from growth factor stimulation requires ROS signaling.

The constitutive activation of this pathway in cancer cells is associated with basal increase in oxidant signaling.

Neoplastic transformation is associated with an increase in the basal level of ROS mediated signaling (Schumaker, 2006).

Chronic increases in ROS may trigger transformation and contribute to cancer progression by amplifying genomic instability.
The warburg effect of aerobic glycolysis is a key metabolic hallmark of cancer (Hsu and Sabatini, 2008).

The increased hexose content in the tumor tissues.

Smith, 2000 - increase in hexokinase activity in breast tumors cases.

Glucokinase and Hexokinase 2 have the ability to suppress apoptosis through the interaction with mitochondria and suppression of Cytochrome C release (Bryson JM et al., 2002).
Glucose-6-phosphatase is a marker enzyme for liver microsomal activity and it is greatly inhibited in cancer bearing animals.

Langeswaram et al., 2013 observed decreased levels of G-6-phosphatase and F-1, 6-bisphosphatase in liver of patients with breast cancer.

This may be due to the flux of intermediates of glycolysis to oxidative and non-oxidative phase of pentose phosphate pathway (De Berardinis et al., 2008).
In cancer tissue the HMP pathway also operates at a high rate.

The oxidative phase of HMP pathway provides the NADPH while the non oxidative phase gives the Ribose-5-Phosphate.

The decreased G-6-P dehydrogenase activity in the cancer tissues might suggest a state in which the cells need for Ribose-5- Phosphate outweighs its need for glucose derived NADPH (De Berardinis et al., 2007).
Tumor cells engaged in this form of metabolism have G-6-P dehydrogenase independent NADPH supply i.e glutamine dependent pathway.

There is also evidence that some glutamine derived carbon can exit TCA cycle as malate and serve as substrate for maleic enzyme which produces NADPH (De Berardinis et al., 2008).

Glutamate can be directly converted into GSH by the enzyme glutathione cysteine ligase (Cairns et al., 2011).
CONCLUSION

- We conclude that the high basal level of oxidative stress with altered enzyme activities of carbohydrate metabolism and G6PD independent supply of reducing equivalents was observed in canine mammary tumor.

- However, much more clinical data will be required in order to discern definite enzyme patterns.
REFERENCES:

Thank you...