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CORRESPONDING AUTHOR

Beatrice Ruggerone

beatrice.ruggerone@unimi.it

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Analytical aspects of protein carbonyl group in oxidized canine serum.

B. Ruggerone^{a*}, G. Colombo^b, S. Paltrinieri^a

^a Department of Veterinary Medicine, University of Milan, Via Celoria, 10 -20133 Milan, Italy

^b Department of Biosciences, University of Milan, via Celoria 26, 20133 Milan, Italy

Abstract

Oxidative stress (OS) is an imbalance between oxidants and anti-oxidants and plays an important role in the aetiology and/or the progression of several diseases. Protein carbonyl (PCO) groups are so far used as biomarkers of OS in humans (Colombo *et al*, 2015).

The aim of our study was to investigate whether PCOs are present in canine serum and if they can be measured spectrophotometrically using a method not yet validated in dogs.

The presence of PCO was investigated by Western blotting after separation by SDS-PAGE of serum at different dilutions (either before or after oxidation with 10% cigarette smoke extract). Protein labelling with 2,4-Dinitrophenylhydrazine (DNPH, Brady's reagent) was followed by a two-steps incubation with primary anti-dinitrophenyl-KLH antibodies (rabbit IgG fraction) and secondary goat anti-rabbit IgG, HRP conjugate. Signal was developed with Enhanced Chemiluminescence (ECL).

Serum PCO were quantified using a commercially available assay (Protein Carbonyl Content Assay Kit - Abcam, UK), based on derivatization of proteins with 1,4-dinitrophenylhydrazine (DNPH) and subsequent formation of protein-conjugated dinitrophenylhydrazones (DNPs) with a peak absorbance at 366 nm.

Western blot showed an evident band of apparent MW of 69 kDa, consistent with carbonylated dog serum albumin. The spectrophotometric assay failed to demonstrate any signal at 366 nm using the manufacturer's instructions or modified protocols.

This study demonstrated that PCO are present in oxidized canine serum. However, the spectrophotometric assay employed in this study is not enough sensitive to detect PCOs. Further studies are needed to assess whether this depends on the poor re-solubilisation of DNPs, or on the low concentration of PCOs in dogs.

References

Colombo, G., Clerici, M., Garavaglia, M.E., Giustarini, D., Rossi, R., Milzani, A., Dalle-Donne, I., 2015. A step-by-step protocol for assaying protein carbonylation in biological samples. *J Chromatogr B*.