Oxidative stress-induced inflammatory responses and effects of N-acetylcysteine in bovine mammary alveolar cells

Hyojin Bae[1], Sukyung Kang[1], Chang Hee Jeong[1], Hong Gu Lee[2], Jeong Tae Do[3], Sung Gu Han[1]*

[1]Toxicology and Milk Science Laboratory, Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Republic of Korea

[2]Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea.

[3]Department of Stem Cell and Regenerative Biology, Konkuk University, Seoul 05029, Republic of Korea

Short title: **Oxidative stress-induced inflammation in MAC-T cells**

*Correspondence: Sung Gu Han, Ph.D.

Department of Food Science and Biotechnology of Animal Resources,

Konkuk University, Seoul 05029, Korea.

Tel: +82-2-450-0526; Fax: +82-2-455-1044

E-mail: hansg@konkuk.ac.kr

Summary

Bovine mastitis causes reduced milk production and poor milk quality. Excessive lactation can produce oxidative stress in the mammary tissue. Excessive lactation is also known to be associated with bovine mastitis, an inflammation of the udder. Thus, in the current study, we hypothesized that oxidative stress increases inflammatory responses in bovine mammary cells. To examine the hypothesis, we produced cellular oxidative stress and investigated resulting inflammatory responses in bovine mammary alveolar cells (MAC-T). To produce oxidative stress, cells were treated with the reactive oxygen species (ROS; e.g., superoxide anion)-producing agent, menadione (MD; 0-10 μM; 6 h). To ensure the ROS-induced responses, cells were pretreated with an antioxidant NAC (0-10 mM; 1 h). Results showed that MD elevated intracellular ROS levels and protein expression of cyclooxygenase-2 (COX-2), a biomarker of inflammation. Pretreatment of cells with NAC attenuated MD-induced COX-2 expression by scavenging intracellular ROS and enhancing intracellular glutathione levels. MD-induced COX-2 expression was mediated by activation of extracellular signal receptor-activated kinase 1/2 (ERK1/2), Akt, and nuclear factor-kappa B (NF-κB). NAC attenuated activation of these intracellular signaling molecules. Treatment of cells with pharmacological inhibitors for ERK1/2, Akt, and NF-κB confirmed the association of these signaling pathways in MD-induced COX-2 expression. These results support our hypothesis that oxidative stress, which is found in over-lactating cows, can produce cellular inflammation in bovine mammary alveolar cells and prevention of oxidative stress can attenuate such pathological responses.