

## Uric Acid as a Modulator of Protein Glutathionylation and Oxidative Stress in Redox Processes in Inflammation

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Uric acid is the end product of purine metabolism. However, the increase in uric acid is associated with inflammatory disorders. Our group demonstrated that uric acid can be oxidized by the inflammatory enzyme myeloperoxidase to produce highly reactive intermediates, such as urate free radical and urate hydroperoxide. Urate hydroperoxide is a thiol and amine oxidant. Our group also demonstrated that uric acid leads to a higher oxidation of glutathione (GSH) in dHL-60 (neutrophil-like) cells activated with PMA, a NOX activator or *Pseudomonas aeruginosa* (PA14). Therefore, we hypothesized that the uric acid could also induce protein glutathionylation, a post-translational modification that can lead to distinct structural and functional changes in the target protein. Besides, S-glutathionylation has the potential to act as a biological switch critical for oxidative signaling events. In this study, dHL-60 cells were activated with PMA or PA14 (MOI 1:10) in the presence or absence of uric acid and the modification on protein by GSH were analyzed using liquid chromatography coupled to mass spectrometry. Our results showed that activation of dHL-60 cells with PA14 or PMA promoted glutathionylation on proteins. In specific, the neutrophilic protein defensin 3 was glutathionylated in all groups tested (non-activate, PMA and PA14 activated neutrophils and upon treatment with uric acid). No exclusive protein was found in non-activated cells. dHL-60 with PA14 and PA14 plus uric acid presented the exclusive glutathionylated proteins cornifina A and eosinophil peroxidase, respectively. Whereas azurocidin and the antimicrobial protein cathelicidin were glutathionylated in both PA14 and PA14 plus uric acid groups. None exclusive glutathionylated proteins were found in neutrophils incubated with PMA. However, five exclusive glutathionylated proteins were found in neutrophils incubated with PMA plus uric acid. Among these, the S100A8 protein (Calprotectin), an important manganese and zinc chelating protein with antimicrobial activity was identified. Therefore, it can be concluded that uric acid, through its oxidative by products, enhances oxidative stress and protein glutathionylation during dHL-60 oxidative burst induced by PA14 or PMA. These findings suggest that uric acid may be involved in cellular signaling mechanisms involved in oxidative stress, which may trigger exacerbation of inflammation. Therefore, this study may shed light on mechanisms involving uric acid and inflammatory diseases.

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