Poster 1

THE EFFECT OF α-1-ACID GLYCOPROTEIN ON ROS PRODUCTION *IN VITRO* IS LIMITED BY STIMULUS NATURE AND SIMULTANEOUS PRESENCE.

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Introduction

Alpha-1-acid glycoprotein (AGP) is an acute phase protein with described anti-inflammatory and immunomodulating properties. AGP is described as a potent inhibitor of neutrophil functions like chemotaxis and production of reactive oxygen species (ROS) in human neutrophils. However published reports about the mechanism of inhibition are conflicting. The influence of bovine AGP on the different stimuli-induced ROS production by bovine peripheral blood polymorphonuclear leukocytes (PMN) was studied using a highly sensitive method approaching its inhibitory mechanism.

Materials and Methods

Bovine peripheral blood PMN were purified by ficoll separation of mononuclear leukocytes and hypotonic lysis of erythrocytes. Three different stimuli - phorbol 12-myristate 13-acetate (PMA), non-opsonized and opsonized *Staphylococcus aureus* bateria were used in different concentrations to induce ROS production in PMN. ROS production was quantified by intracellular oxidation of dihydrorhodamin 123 (DHR) and evaluated by flow cytometry. AGP was tested over a wide concentration range in various experimental set-ups.

Results

AGP efficiently suppressed PMA, but not bacteria or opsonized bacteria-induced ROS generation *in vitro*. The suppressive effect was concentration-dependent and adversely proportional to PMA concentration. The selective inhibitory potential of AGP in comparison with ovalbumin and bovine serum albumin showed that ROS inhibition was not mere protein effect. ROS production was suppressed only if AGP and PMA were simultaneously present with PMN. Pre-incubation of PMN with AGP did not alter the PMN response to PMA. Moreover, AGP could not suppress ROS production after pre-stimulation of PMN with PMA.

Conclusion

The AGP does not modulate neutrophil responsiveness to stimulus and ROS production directly, but likely blocks PMA, thus reducing the stimulus concentration available for triggering the PMN.