Effect of morphine and propofol on the activation of equine neutrophils : An EPR spin trapping study

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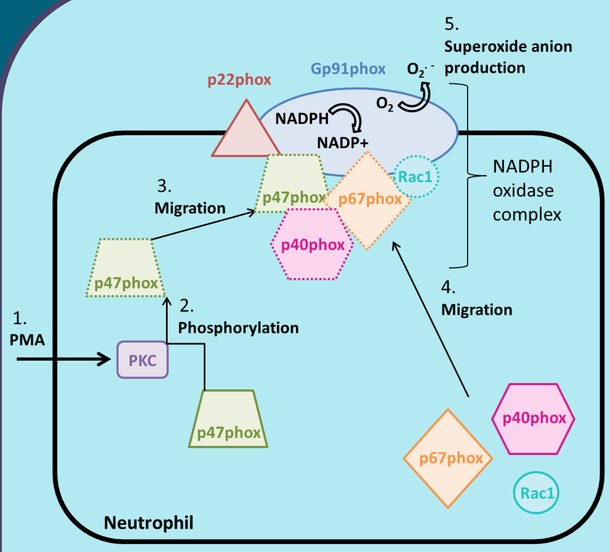


Fig. 1 Proposed mechanism of the membrane NADPH oxidase complex activation and production of the superoxide anion, precursor of ROS, in a PMA-activated neutrophil leukocyte.

Introduction

Inflammation is a complex phenomenon involving chemical and enzymatic mechanisms. The polymorphonuclear neutrophil leukocytes (PMNs) play an essential immunomodulatory role by releasing harmful reactive oxygen species (ROS) and oxidant enzymes. During the respiratory burst, the enzymatic complex NADPH oxidase produces the ROS precursor: superoxide anion ($O_2^{\cdot -}$), to initiate the invasive microorganisms degradation. Inflammatory pathologies induce an excessive stimulation of the neutrophils and an uncontrolled production and release of ROS in the extracellular medium, leading to severe tissue damages. The treatment of chronic pathologies is still a challenge to rise. For this reason, the research of potential anti-inflammatory molecules, able to inhibit the oxidant enzymes and/or to scavenge ROS, is an important part of biomedical research. Morphine and propofol, in addition to their analgesic and anaesthetic action respectively, presents antioxidant properties [3,4]. However, only few data has been reported on their potential superoxide anion scavenger and inhibitory effect on the neutrophil respiratory burst.

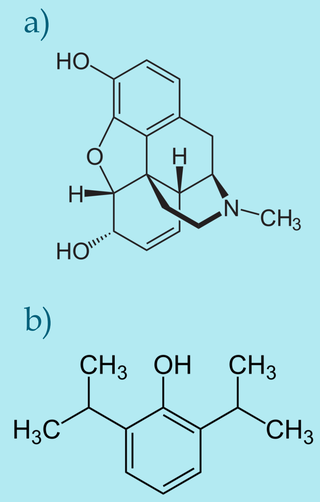


Fig. 2 Structure of a) morphine and b) propofol

Aims of the study

- Study of the reducing action of morphine and propofol on superoxide anions produced by both enzymatic and cellular systems.
- Comparison between the action of both interest molecules and two polyphenols, quercetin and gallic acid, by EPR spectroscopy using spin trapping technique.

Methods and results

EPR study on the enzymatic xanthine / xanthine oxidase system

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid} + O_2^{\cdot -} + H^+$$

$$\text{DMPO} + O_2^{\cdot -} \longrightarrow \text{DMPO} - \text{OOH}$$

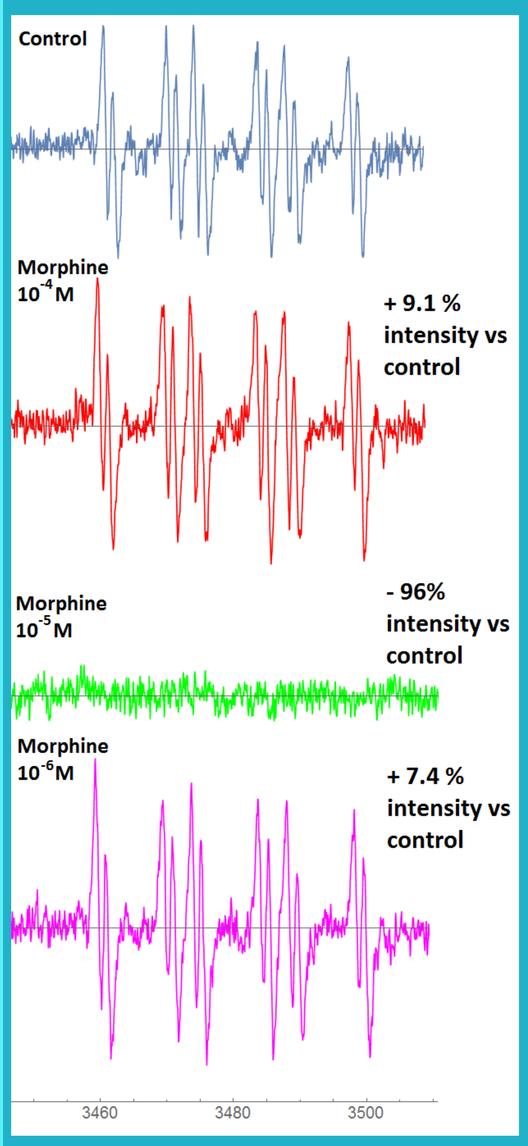


Fig. 3 Action of morphine on the superoxide anions formed by the xanthine - xanthine oxidase system. Observation of the scavenging action resulting in the inhibition of the DMPO-OOH adduct formation in the presence or absence of drug. [X] = M, [XO] = U/ml, [DMPO] = M, [DTPA] = M, in phoshpate buffer pH 7.4

Morphine decreases the intensity of the DMPO-OOH adduct spectrum by 96% at 10^{-5}M. The molecule can act as a superoxide scavenger. However, this action vary with contentration. An increase of superoxide anions production can be observed at lower and higher concentrations.

EPR study on the cellular system

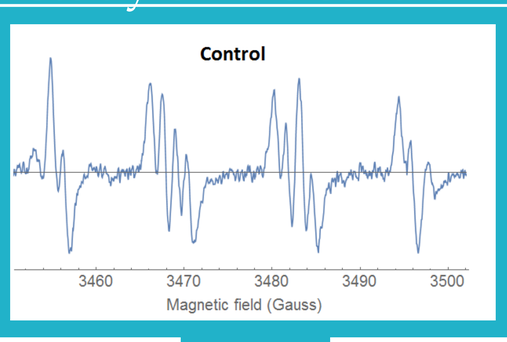
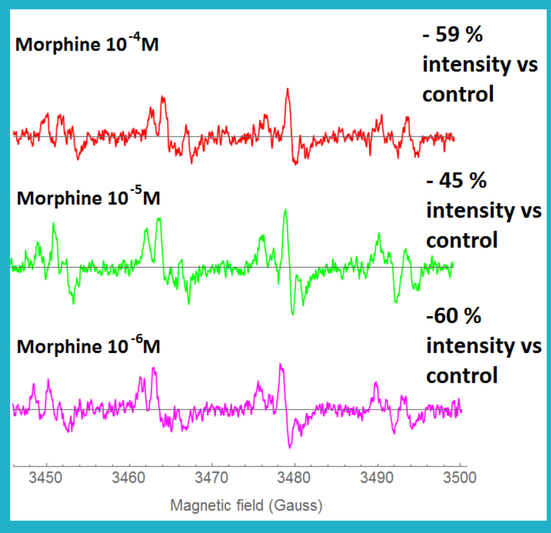
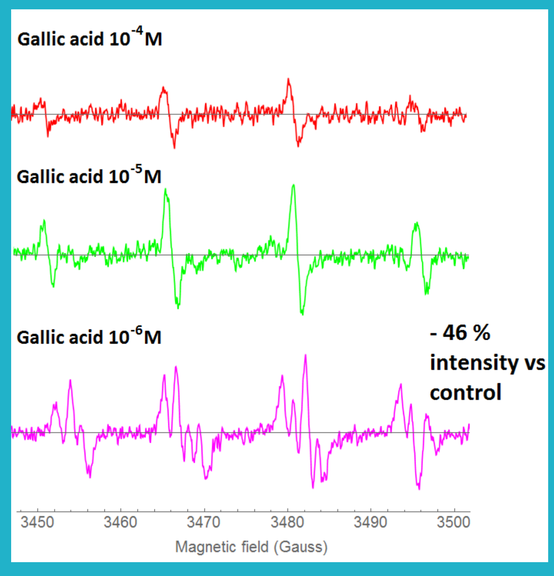


Fig. 4 Action of morphine, propofol quercetin and gallic acid on the superoxide anions formed by the stimulated equine polymorphonuclear neutrophil leukocytes. Observation of the scavenging action resulting in the inhibition of the DMPO-OOH adduct. formation. [PMNs] = 10^6/100µl, [Ca] = M , [DMPO] = M, [PMA] = M, in HBSS buffer pH 7.4





Pravastatin induces the concentration-dependent decrease of the DMPO-OOH adduct, by the scavenging ot the superoxide anions produced by the cellular system. However, pravastatin clearly demonstrates a weaker action than the one of a polyphenol, quercetin, which totally inhibits the production of DMPO-OOH at all concentrations. The concentrations of pravastatin used exceed the clinical ones, which are generally between 5 10^{-5} and 10^{-6} M. The goal of this EPR study was to highlight the statin potential ROS scavenging activity and therefore its antioxidant action. Luminescence studies, realized in parallel of this EPR study, showed the scavenging action of pravastatin at low concentration.

Conclusion

The ROS scavenger action of morphine and propofol towards superoxide anions, the ROS precursor produced during the neutrophil respiratory burst, initiating the inflammation process, has been evidenced on two models (enzymatic and cellular) using EPR spin trapping technique. In both case, pravastatin exhibits a dose-dependent scavenger activity. However, the statin action is weaker than the one of a reference polyphenolic antioxidant, quercetin which totally suppress the DMPO-OOH EPR signal at all tested concentrations. Overall, the EPR results indicate that pravastatin might have an interest for the treatment of inflammatory pathologies by decreasing the production of $O_2^{\cdot -}$.

References

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