Study of the antioxidant action of two analgesic compounds, morphine and propofol, on the activity of MPO and HRP

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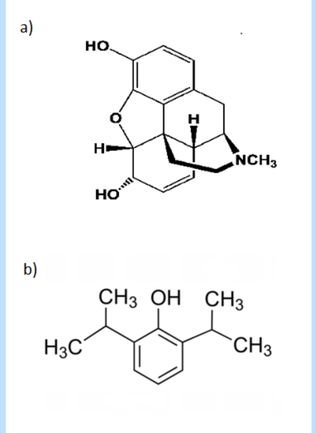
Introduction

Although acute inflammation can efficiently be treated, the treatment of chronic inflammatory pathologies is still a challenge to rise. As marker of inflammation, the enzyme Myeloperoxidase (MPO) is a choice target for the establishment of a treatment. Indeed, the uncontrolled release in the extracellular medium of MPO, marker of inflammation, along with the release of ROS, causes severe damages on biological tissues. The modulation of the enzyme activity, by an inhibitor, might constitute an approach to treat excessive inflammation. An interesting pathway is to give a second life to clinical-used molecules, presenting antioxidant and anti-inflammatory properties. According to several studies, morphine and propofol (PPF), which are already known for their analgesic and anesthetic properties, present an antioxidant activity. Therefore, these reducing molecule can potentially pretend to be MPO inhibitors.

Fig.1: Chemical structure of a) morphine b) propofol



Aims of the study

- Evaluate the potential reducing and anti-catalytic actions of morphine and PPF on the similar peroxidase activity of two enzymes : MPO and HRP, with two complementary techniques: EPR and docking
- Compare the activity of morphine and PPF *versus* two polyphenols, quercetin and gallic acid, and ascorbic acid

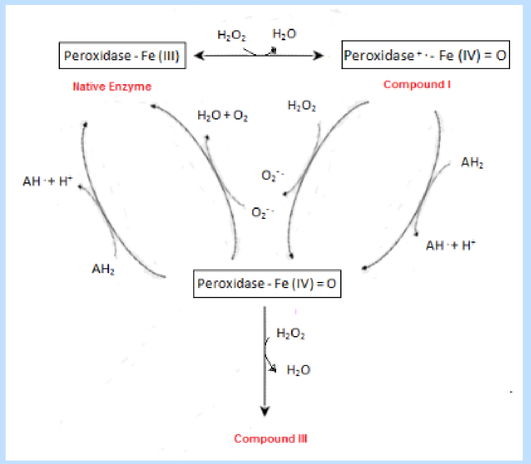


Fig.2: Scheme of the peroxydase cycle of a peroxidase enzyme triggered by the interaction with its natural substrate H_2O_2 or with a reducing substrate AH_2

Methods and results

EPR study

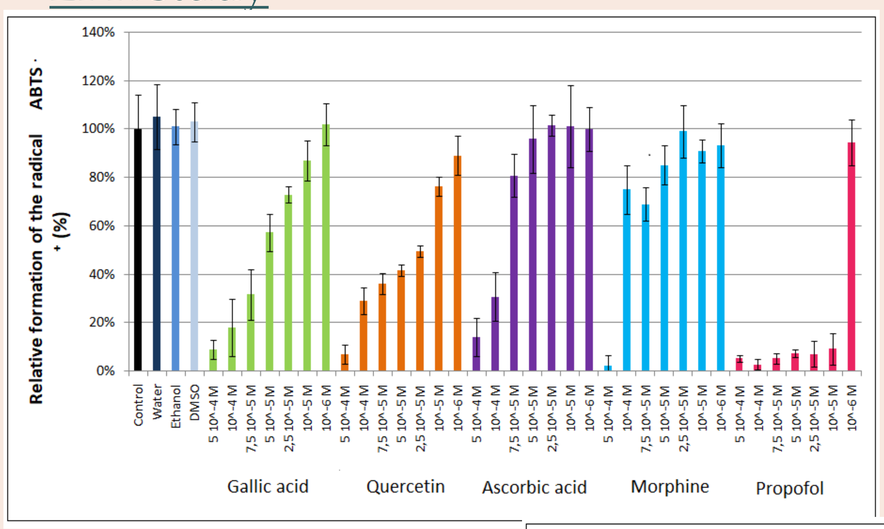


Fig.3: Action of the antioxidant compounds on the $ABTS^{\cdot+}$ radical. The radical is formed by the oxidation of ABTS by the peroxidase cycle of HRP.
The percentages of inhibition of MPO activity for each molecules were calculated versus their respective solvent control. Data are given as mean ± SD (n≥ 5). ([HRP]=3,41 10^{-7} M, $[H_2O_2]$=4,85 10^{-5} M, [ABTS]=6,75 10^{-5} M, in phosphate buffer pH 7.4).

Fig.4: Action of the antioxidant compounds on the$ABTS^{\cdot+}$ radical, after 30 minutes.
The percentages of inhibition of MPO activity for each molecules were calculated versus their respective solvent control. Data are given as mean ± SD (n≥ 5). ([HRP]=3,41 10^{-7} M, $[H_2O_2]$=4,85 10^{-5} M, [ABTS]=6,75 10^{-5} M, in phosphate buffer pH 7.4).

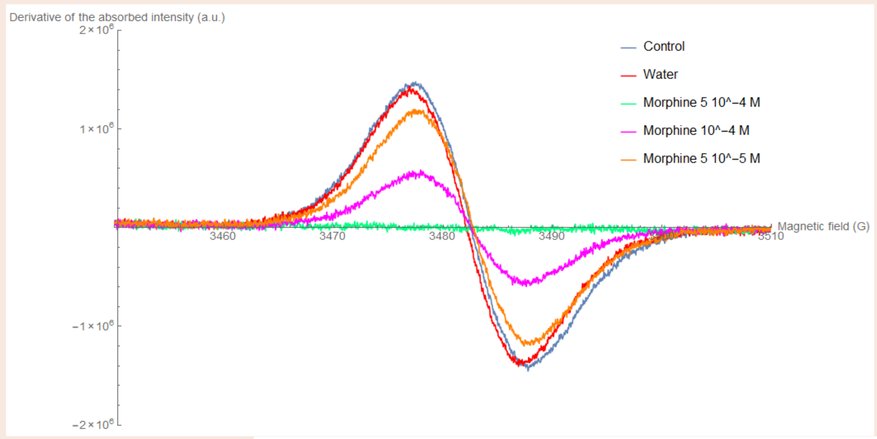


Fig. 5: Protective action of morphine against the oxidation of ABTS by the peroxidase cycle of MPO.
([MPO]=1.7 10^{-2}M, $[H_2O_2]$=2.410^{-4}M, $[NaNO_2]$=5 10^{-3} M, [ABTS]=3.74 10^{-4} M).

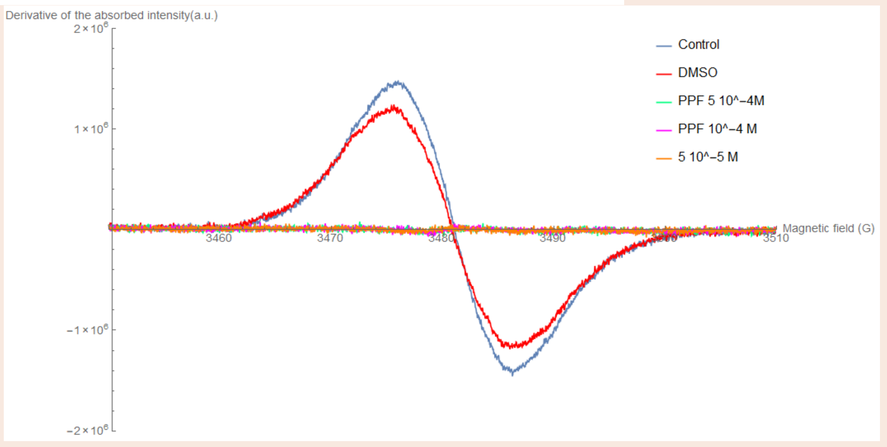
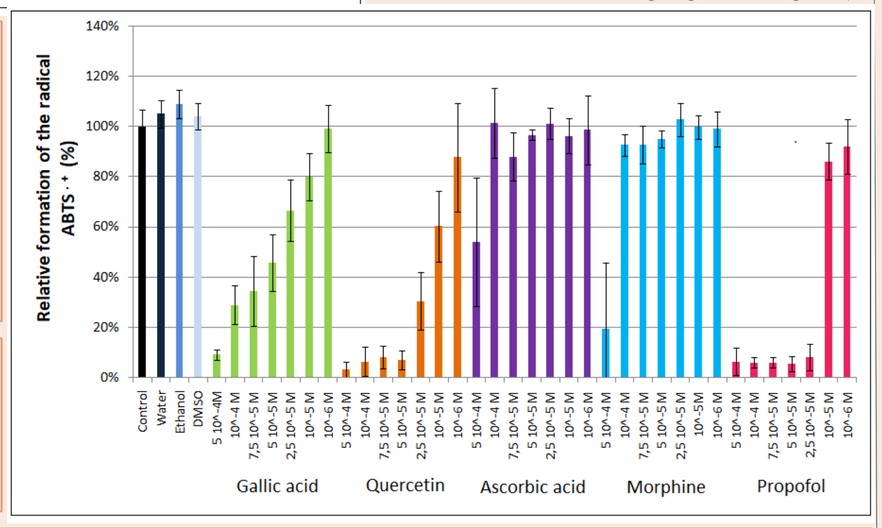


Fig. 6: Protective action of propofol against the oxidation of ABTS by the peroxidase cycle of MPO.
([MPO]=1.7 10^{-2}M, $[H_2O_2]$=2.410^{-4}M, $[NaNO_2]$=5 10^{-3} M, [ABTS]=3.74 10^{-4} M).

Gallic acid and quercetin exhibit a dose-dependent action on the formation of the ABTS radical state. The association of their anti-catalytic and their important reducing actions, due to their number of hydroxyl groups and their stabilization by electronic resonance, allow them to increase their action with time.

Morphine and ascorbic acid cause a reduction of the $ABTS^{+\cdot}$ formation at high concentration. After 30 minutes, no significant effect can be observed, except at 5 10^{-4} M.



The presence of propofol decrease the $ABTS^{+\cdot}$ concentration by more than 90 %, until the lowest concentration 10^{-6} M. The action of PPF is an association of a reductive action on $ABTS^{+\cdot}$ and on the HRP cycle. This activity is stable in time thanks to the electronic stabilization of the PPF radical state. However, propofol loses 77% of its action at 10^{-5} M.

Morphine and propofol act as reductive substrates in the peroxidase cycle of MPO. They enter in competition with ABTS and therefore inhibit its oxidation by compound I and compound II. The activity of morphine is dose-dependent, in contrast to PPF, which inhibits totally the formation of the radical $ABTS^{+\cdot}$ at all three concentrations. These results shows the ability of morphine and PPF to enter the active site of MPO, to interact with the enzyme peroxidase intermediates. Therefore, a part of both molecules can potentially be attributed to their steric effect inside the heme cavity.

Docking study

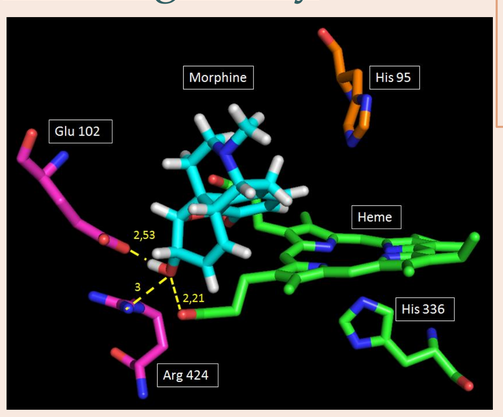


Fig. 7: the solution obtained by the docking of morphine in the active site of MPO. Docking program: GOLD

The 3D structure of morphine doesn't allow the molecule to enter deep in the active site of MPO, to bond with important amino acids, like His 95 and therefore to inhibits the enzyme catalytic action.

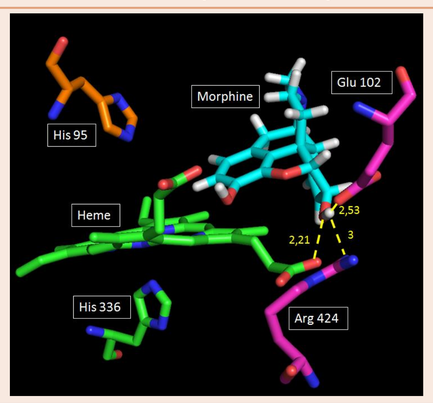
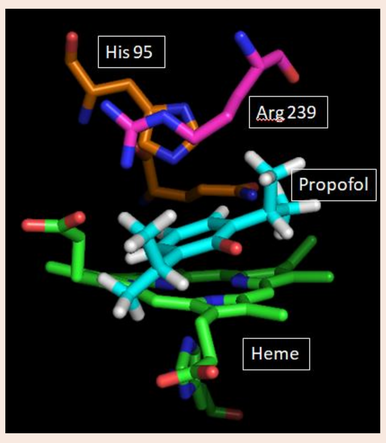
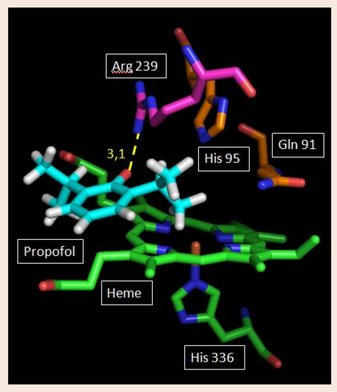


Fig. 8: The two solutions obtained by the docking of propofol in the active site of MPO. Docking program: GOLD





The small size and the planar structure of propofol allows the molecule to enter the heme cavity and to get close to the heme. This observation is in agreement with the EPR results, demonstrating the potent reductive action on MPO peroxidase cycle. However, the two dimethyl groups don't allow the molecule to be correctly positioned to build bonds with important active site amino acids, like His 95, which intervenes in the trigger of MPO peroxidase cycle. Therefore, PPF doesn't seem to be able to present an anti-catalytic action.

Conclusion

EPR spectroscopy allows to demonstrate the antioxidant property of propofol and morphine. These two molecules react as reductive substrate in the peroxidase cycle of both enzymes, MPO and HRP. Indeed, the association of EPR and molecular modeling confirms the ability of the molecules to enter peroxidase active site. However, the docking study suggests the absence of strong bonds with important amino acids and therefore of anti-catalytic action. This hypothesis has to be confirmed via the SIEFED technique. The stronger action of PPF versus morphine can partially be attributed to the greater electronic stabilization of the PPF radical state. However, the absence of a dose-dependent action has still to be explained.

References

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