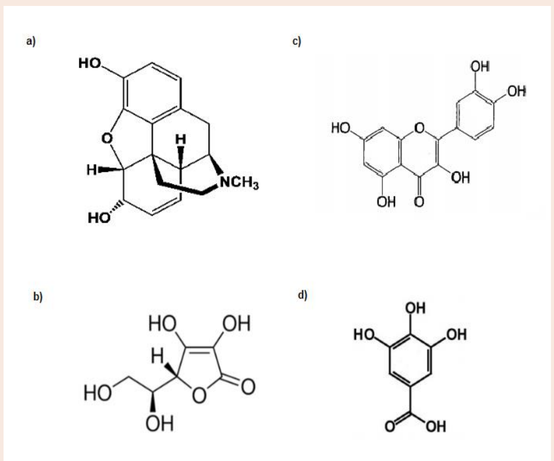
Comparison of the action of morphine with potential inhibitors on the activity of MPO and HRP

P. Nyssen[a], T. Franck[b], E. Sauvage[c], A. Mouithys-Mickalad[b] , M. Hoebeke [a]

[a] Biomedical Spectroscopy, CESAM, Faculty of Sciences, B 5a , University of Liège - Sart-Tilman, Liège, Belgium, [b]Centre of Oxygen, Research and Development, Institute of Chemistry B 6a, University of Liège - Sart Tilman, Liège, Belgium, [c] Center for Protein Engineering , Faculty of Sciences, B 5a , University of Liège - Sart-Tilman, Liège, Belgium

Fig.1: Chemical structure of a) morphine b) ascorbic acid c) quercetin d) gallic acid



Introduction

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 might constitute an approach for the treatment of excessive inflammation. According to several studies, morphine, which is already known for its analgesic properties, seems to present an antioxidant activity. Therefore, this reducing molecule can potentially pretend to be an inhibitor of MPO.

Aims of the study

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

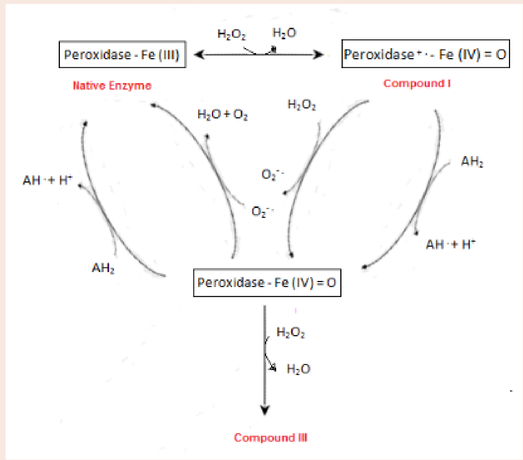


Fig.2: Scheme of the peroxydase cycle of a peroxidase enzyme triggered by the interaction with its natural substrate H_2O_2 or with a reducing substrate AH_2

Methods and results

EPR study

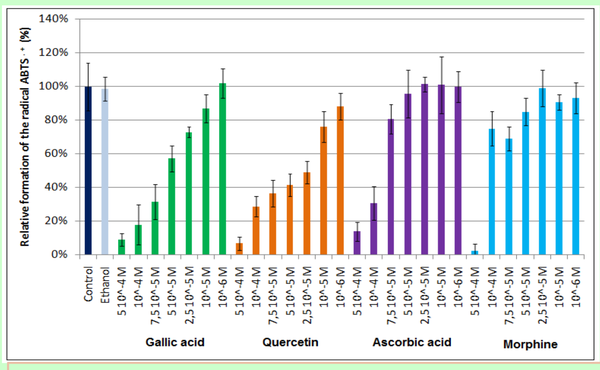
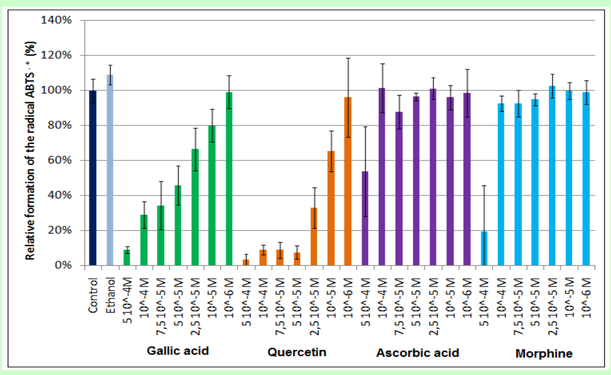


Fig.3: Action of the antioxidant compounds on the ABTS·+ radical. The radical is formed by the oxidation of ABTS by the peroxidase cycle of HRP. The percentages of inhibition of MPO activity for each molecules were calculated versus their respective solvent control. Data are given as mean ± SD (n≥ 5). ([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).

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.

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).



SIEFED study

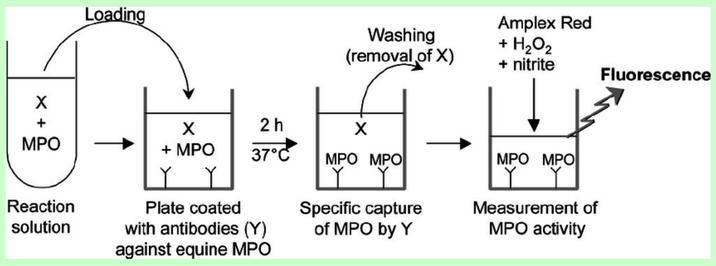


Fig.5: Scheme illustrating the SIEFED method

Gallic acid and quercetin totally inhibit the MPO activity, except at the lowest concentration (10^{-6} M). The SIEFED results indicate that their action is due to the ability of these two polyphenols to enter the active site of MPO and to establish bonds with amino acids, which resist to the washing step of the experiment. Morphine does not exhibit any significant anti-catalytic action on MPO. However, Fig. 7 shows that morphine presents a reducing action on the intermediates of the MPO peroxidase cycle. The competition between the molecule and amplex red, as substrate for the cycle, prevents the oxidation of the latter and reduces the fluorescence signal. As morphine, ascorbic acid has a less efficient action on the MPO activity than the two polyphenols. This action seems to be due to the association of both reducing and anti-catalytic actions, the latter allowing an inhibition of around 90 % of MPO activity at 10^{-4} M.

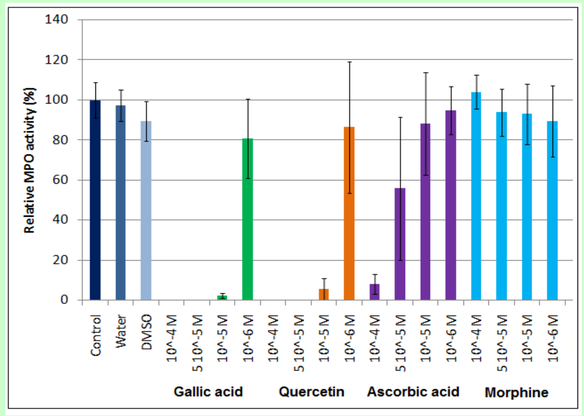


Fig.6: Effect of the compounds on the MPO activity measured by the SIEFED method. The anti-catalytic action on MPO is studied. The percentages of inhibition of MPO activity for each molecules were calculated versus their respective solvent control. Data are given as mean ± SD (n≥ 5).

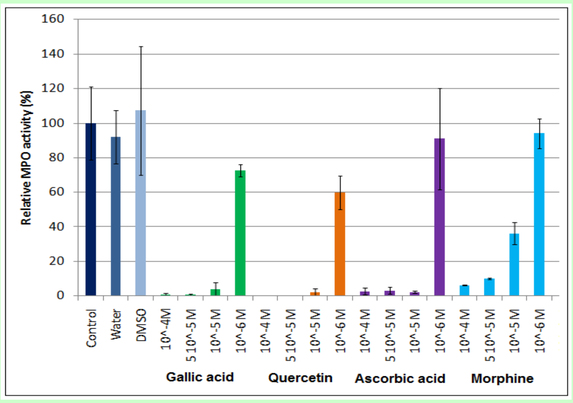


Fig.7: Effect of the compounds on the MPO activity (SIEFED without the washing step). The association of the anti-catalytic action and the reducing action on the peroxidase cycle of MPO is studied. The percentages of inhibition of MPO activity for each molecules were calculated versus their respective solvent control. Data are given as mean ± SD (n≥ 5).

Docking study

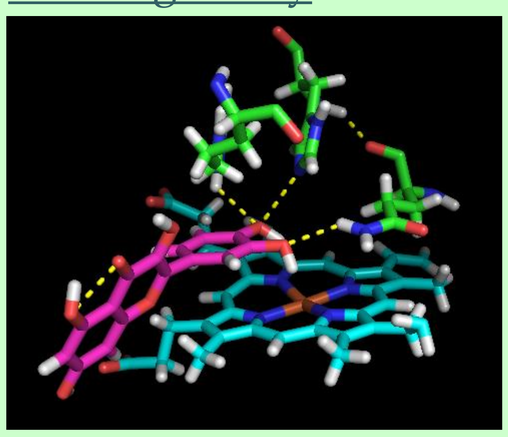


Fig. 8:A solution obtained by the docking of quercetin in the active site of MPO: heme in blue, His 95, Gln 91, Arg 239 in green and quercetin in pink. (Docking program: Yasara)

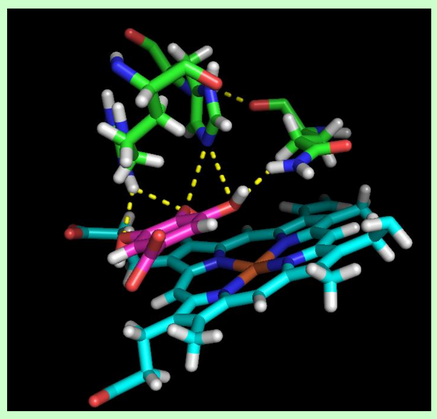


Fig. 9: A solution obtained by the docking of gallic acid in the active site of MPO: heme in blue, His 95, Gln 91, Arg 239 in green and gallic acid in pink. (Docking program: Yasara)

The results obtained by docking are in accordance with the SIEFED ones. Quercetin and gallic acid can easily enter the active site of MPO and establish bonds with three important amino acids, taking part in the interaction of MPO with its natural substrate H_2O_2. The aromatic cycle of the molecules are positioned parallel to the heme, preventing the access of H_2O_2.

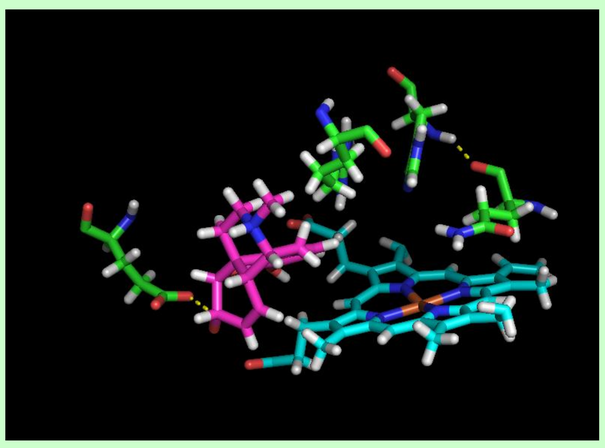
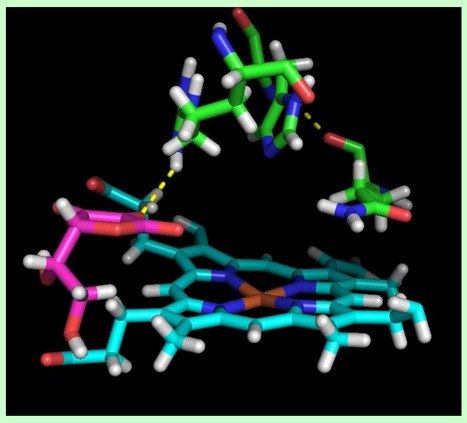


Fig. 10: A solution obtained by the docking of morphine in the active site of MPO: heme in blue, His 95, Gln 91, Arg 239 in green and morphine in pink. (Docking program: Yasara)

Fig. 11: A solution obtained by the docking of ascorbic acid in the active site of MPO: heme in blue, His 95, Gln 91, Arg 239 in green and ascorbic acid in pink. (Docking program: Yasara)



The 3D structure of morphine doesn't allow the molecule to enter the active site of MPO. The molecule does not seem to establish bonds with the enzyme, what would explain why all the morphine molecules are eliminated during the washing step of the SIEFED experiment. In the case of ascorbic acid, the chain attached to the cycle prevents the molecule to correctly enter in the active site but the molecule could also be able to link to Arg 239.

Conclusion

Quercetin and gallic acid, two polyphenols, exhibit a strong inhibition of the MPO activity thanks to their anti-catalytic action. In contrast , morphine seems to have difficulties to enter the active site of the enzyme, because of its structure. However, morphine acts as reducing substrate in the peroxidase cycle of MPO and exhibits an important reducing action at high concentration, similarly to ascorbic acid. The instability of their radical's reducing action over time is due to the low stability by electronic resonance of their radical state.

References

1. S. J. Klebanoff, Myeloperoxidase: friend and foe, Journal of Leukocyte Biology, 77, 598-625, 2005

2. I. Gülçin , S. Beydemir , H. A. Alici ,M. Elmastas M. E. Büyükokurôglu, In vitro antioxidant properties of morphine, Pharmacological Research, 49, 59-66, 2004

3. T. Franck, S. Kohnen, G. Deby-Dupont, S. Grulke, C. Deby, D. Serteyn, A specific method for measurement of equine active myeloperoxidase in biological samples and in in vitro tests, J. Vet. Diagn. Invest., 18, 326-334, 2006

4. T. Franck, A. Mouithys-Mickalad, T. Robert, G. Ghitti, G. Deby-Dupont, P. Neven, D. Serteyn, Differentiation between stoichiometric and anticatalytic antioxidant properties of benzoic acid analogues: A structure redox potential relationship study, Chemico-Biological Interactions, 206, 194-203, 2013

5. Y. Shiba, T. Kinoshita, H. Chuman, Y. Taketani, E. Takeda, Y. Kato, M. Naito, K. Kawabata, A. Ishisaka, J. Terao, Y. Kawai, Flavonoids as substrates and inhibitors of myeloperoxidase: molecular actions of aglycone and metabolites, Chem. Res. Toxicol., 21 (8), 1600-1609, 2008