



THE COMBINATION OF MOLECULAR HYDROGEN AND HEME OXYGENASE 1 EFFECTIVELY INHIBITS NEUROPATHY CAUSED BY PACLITAXEL IN MICE

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INTRODUCTION

Chemotherapy-provoked peripheral neuropathy and the associated affective disorders are important adverse effects in cancer patients, and its treatment is not completely resolved. A recent study reveals a positive interaction between hydrogen rich water (HRW) and a heme oxygenase (HO-1) enzyme inducer, cobalt protoporphyrin IX (CoPP), in the inhibition of neuropathic pain provoked by nerve injury. Nonetheless, the impact of CoPP co-administered with HRW on the allodynia and the emotional disorders related to paclitaxel (PTX) has not yet assessed.

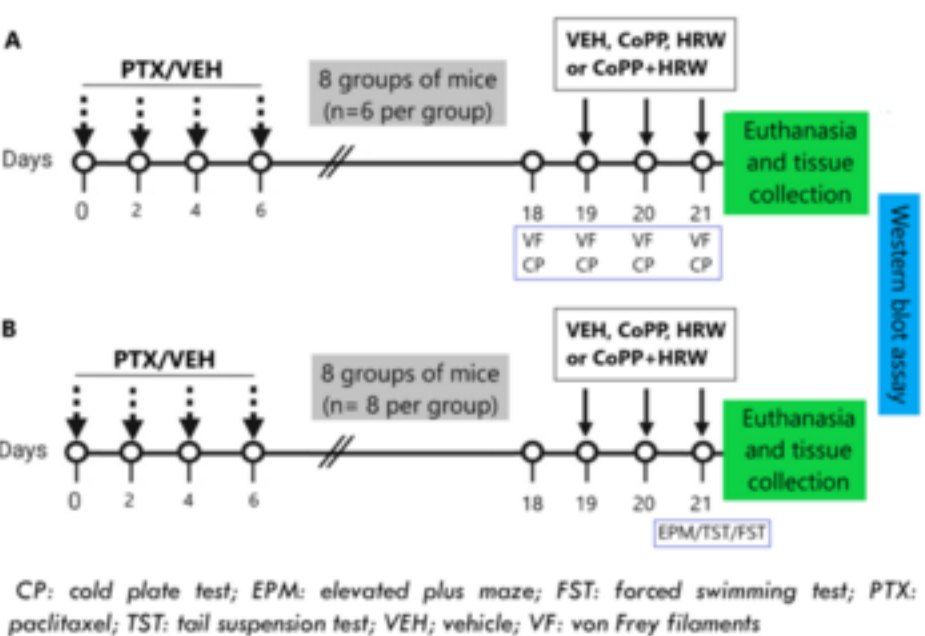
OBJECTIVES

To evaluate: 1) the effects of the co-administration of CoPP and HRW on the allodynia and affective behaviors-linked with PTX and 2) the possible modulator actions of this co-treatment on the inflammatory and oxidative responses caused by PTX in the dorsal root ganglia (DRG) and amygdala (AMG).

MATERIAL AND METHODS

In male C57BL/6 mice i.p. injected with PTX (2mg/kg; diluted in Cremophor EL/ethanol/saline; 1:1:18), every other day, for four successive days, we examined the effects of the i.p. administration of 2.5 mg/kg of CoPP and 0.15 mM of HRW, alone and combined, during three consecutive days on: 1) mechanical and thermal allodynia at days 19, 20, and 21 after PTX injection using von Frey filaments and cold plate test; 2) their effects on the anxiety- and depressive-like behaviors at day 21 after PTX injection using the elevated plus maze, tail suspension and forced swimming tests and 3) the impact of this combined treatment on the protein levels of NLRP3, 4-HNE, NRF2, HO-1, GSTM1 and SOD-1 on the DRG and AMG by western blot (Fig. 1).

Figure 1. Schematic design to evaluate the effects of the i.p. administration of CoPP (2.5 mg/kg), HRW (0.15 mM) or CoPP (2.5 mg/kg) plus HRW (0.15 mM), twice a day, during three consecutive days, on the nociceptive responses (A) and emotive disorders (B) caused by PTX.

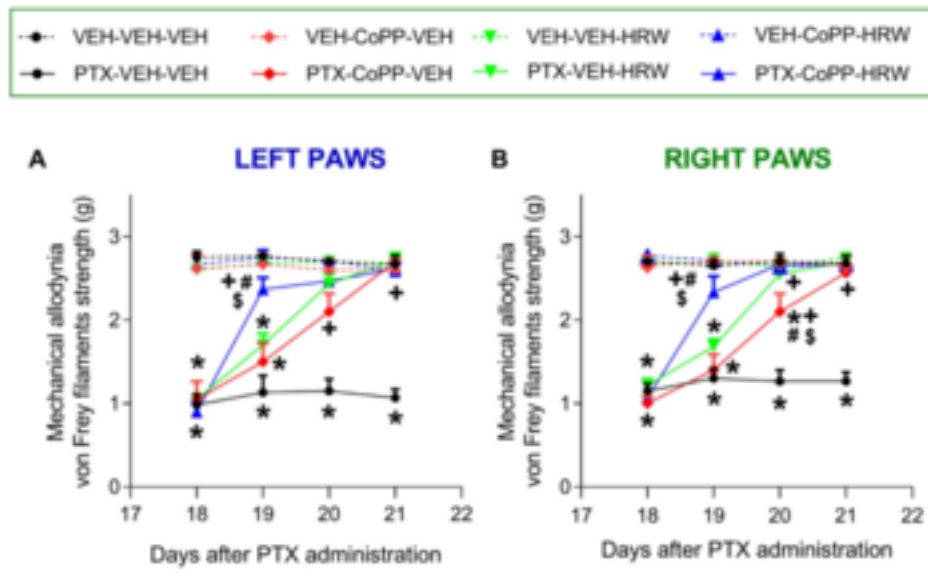


RESULTS

Our results revealed that the antiallodynic actions of the co-administration of CoPP plus HRW are faster and higher than those produced by each of them administered alone (Fig. 2 & 3).

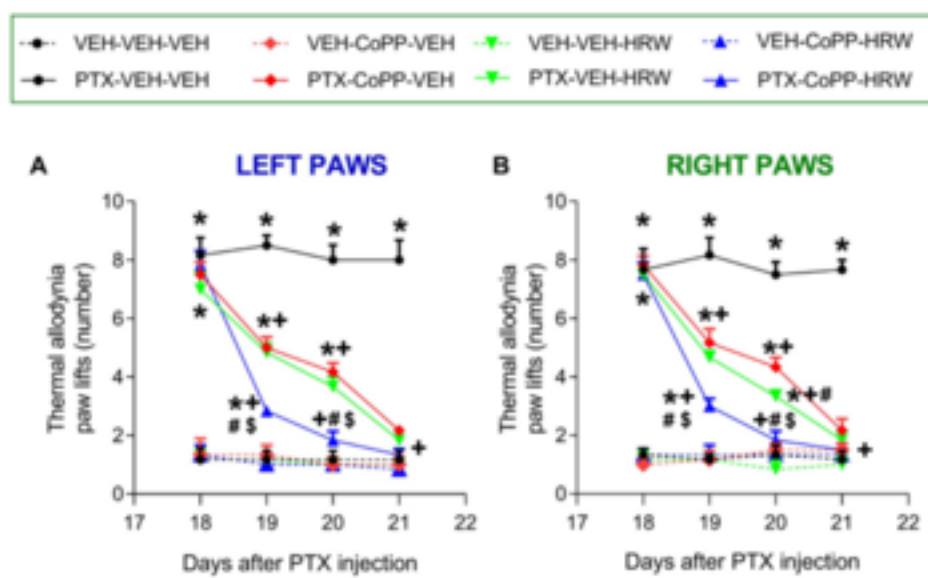
This combination likewise inhibited the anxiodepressive-like behaviors related to PTX (Fig. 4 & 5). This combination also normalized the up-regulation of inflammasome NLRP3 and 4-hydroxynonenal (oxidative stress marker) and stimulated the expression of the antioxidant enzymes Nrf2, HO-1, SOD-1 and GSTM1, in the DRG (Fig. 6) and/or AMG (Fig. 7). Thus, showing a positive interaction among HO-1 and molecular hydrogen in controlling PTX-induced neuropathy by modulating inflammation and activating the antioxidant system.

Figure 2. The inhibition of the mechanical allodynia produced by the i.p. administration, at 2 times per day, of CoPP (2.5 mg/kg) and HRW (0.15 mM), alone and combined, from days 19 to 21 after PTX injection are represented. Data are presented as the von Frey filaments strength (g) on the left (A) and right (B) paws.



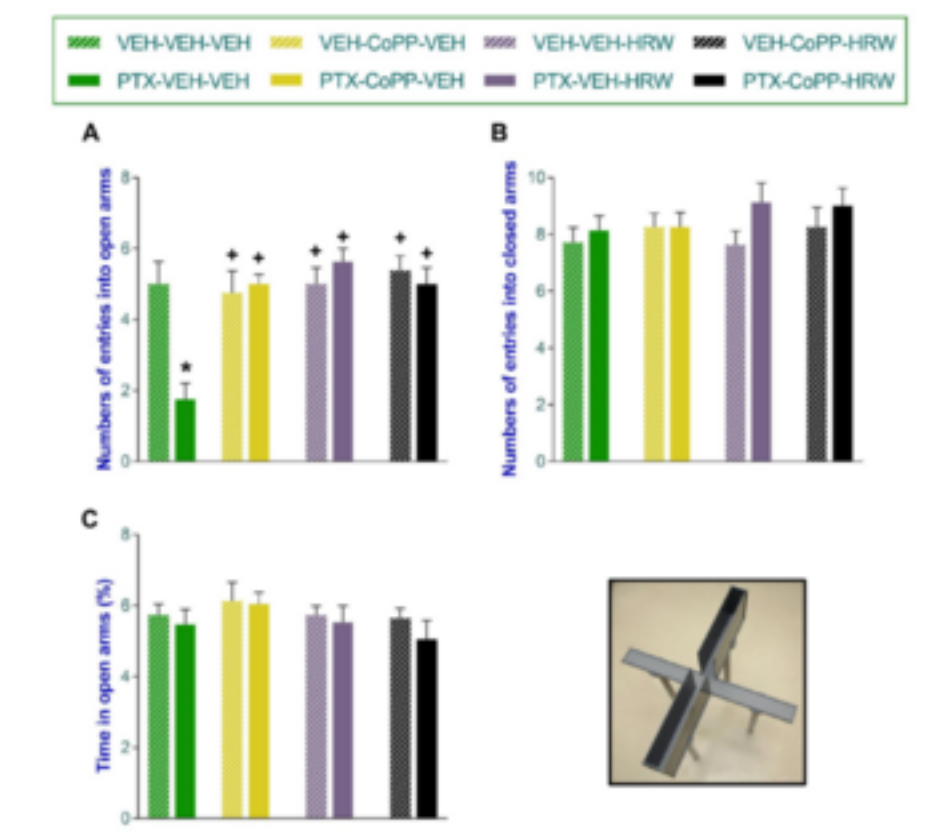
In all tests, symbols show significant differences vs. subjects given * VEH-VEH-VEH, VEH-CoPP-VEH, VEH-VEH-HRW or VEH-CoPP-HRW, + vs. PTX-VEH-VEH, # vs. PTX-CoPP-VEH and \$ vs. PTX-HRW-VEH (p < 0.05, one-way ANOVA and Tukey test). Mean values ± SEM of 6 animals/group.

Figure 3. The inhibition of thermal allodynia produced by the i.p. administration, at 2 times per day, of CoPP (2.5 mg/kg) and HRW (0.15 mM), alone and combined, from days 19 to 21 after PTX injection are represented. Data are presented, as the paw lifts (number) in the left (A) and right (B) paws.



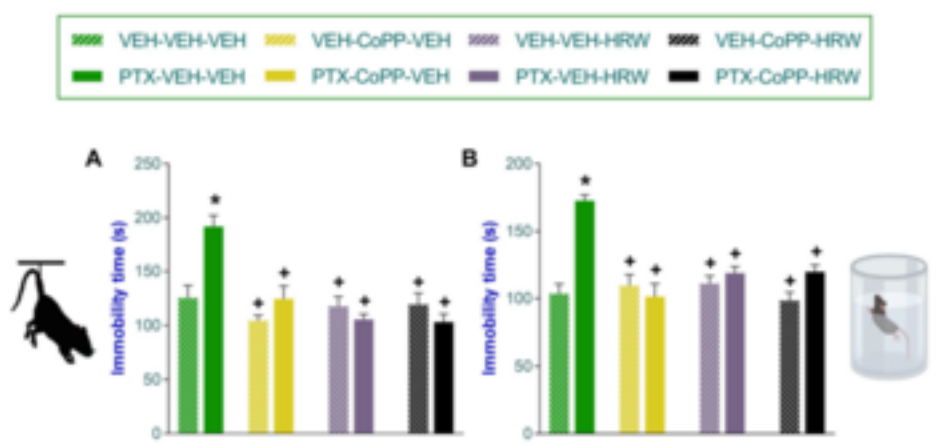
In all tests, symbols show significant differences vs. subjects given * VEH-VEH-VEH, VEH-CoPP-VEH, VEH-VEH-HRW or VEH-CoPP-HRW, + vs. PTX-VEH-VEH, # vs. PTX-CoPP-VEH and \$ vs. PTX-HRW-VEH (p < 0.05, one-way ANOVA and Tukey test). Mean values ± SEM of 6 animals/group.

Figure 4. The inhibition of the anxiety-like behaviors associated with PIPN induced by the i.p. administration of CoPP (2.5 mg/kg) or HRW (0.15 mM), alone and combined, given 2 times per day during three consecutive days, are represented. The effects of CoPP, HRW, CoPP plus HRW or VEH in animals given VEH are also shown. The number of entrances to the open arms (A) and closed arms (B) and the proportion of time passed in the open arms (C) of the EPM are represented.



In all graphs, * signifies significant differences vs. animals treated with VEH-VEH-VEH and + vs. animals treated with PTX-VEH-VEH (p < 0.05, one-way ANOVA and Tukey test). Data are expressed as mean values ± SEM of 8 animals/group

Figure 5. The inhibition of the depressant-like behaviors associated with PIPN induced by the i.p. administration of CoPP (2.5 mg/kg) or HRW (0.15 mM), alone and combined, given 2 times per day, during three consecutive days. The effects of CoPP, HRW, CoPP plus HRW or VEH in animals given VEH are also shown. In the TST (A) and FST (B), the time that the animals remain immobile (s) is represented.



In both graphs, * signifies significant differences vs. animals treated with VEH-VEH-VEH and + vs. animals treated with PTX-VEH-VEH (p < 0.05, one-way ANOVA and Tukey test). Data are expressed as mean values ± SEM of 8 animals/group.

Figure 6. Effects of CoPP combined with HRW on the expression of NLRP3, 4-HNE, NRF2, HO-1, GSTM1 and SOD-1 in the DRG of PTX-injected mice. This combined treatment reversed the up-regulation of NLRP3 (A) and 4-HNE (B) and increased the protein levels of NRF2 (C), HO-1 (D) and SOD-1 (F) in DRG of PTX-injected mice. No changes in the GSTM1 levels (E) were observed.

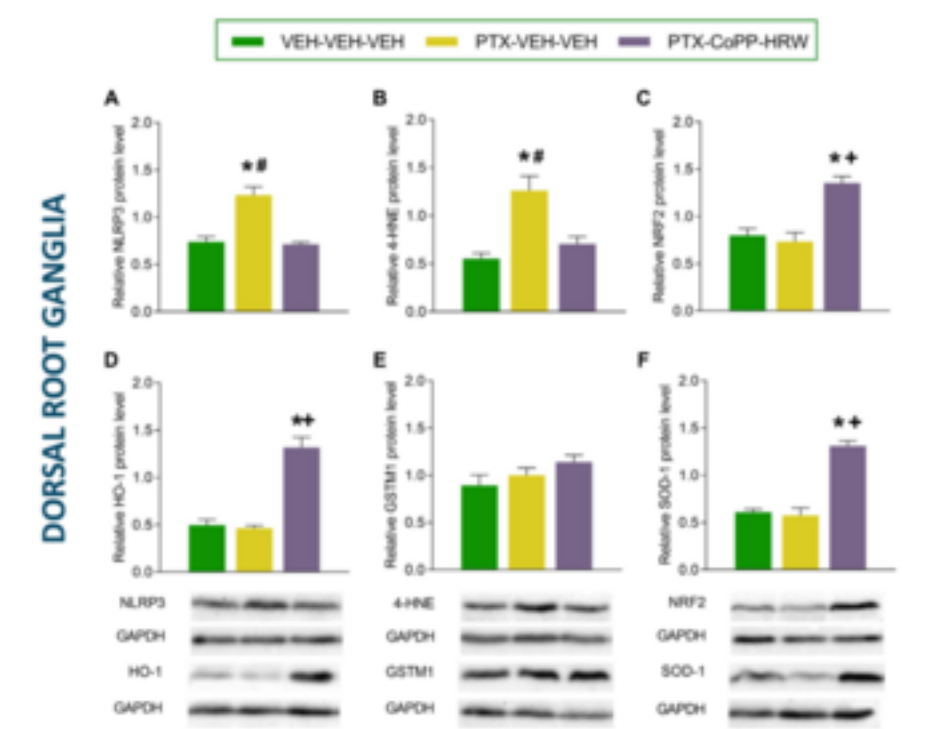
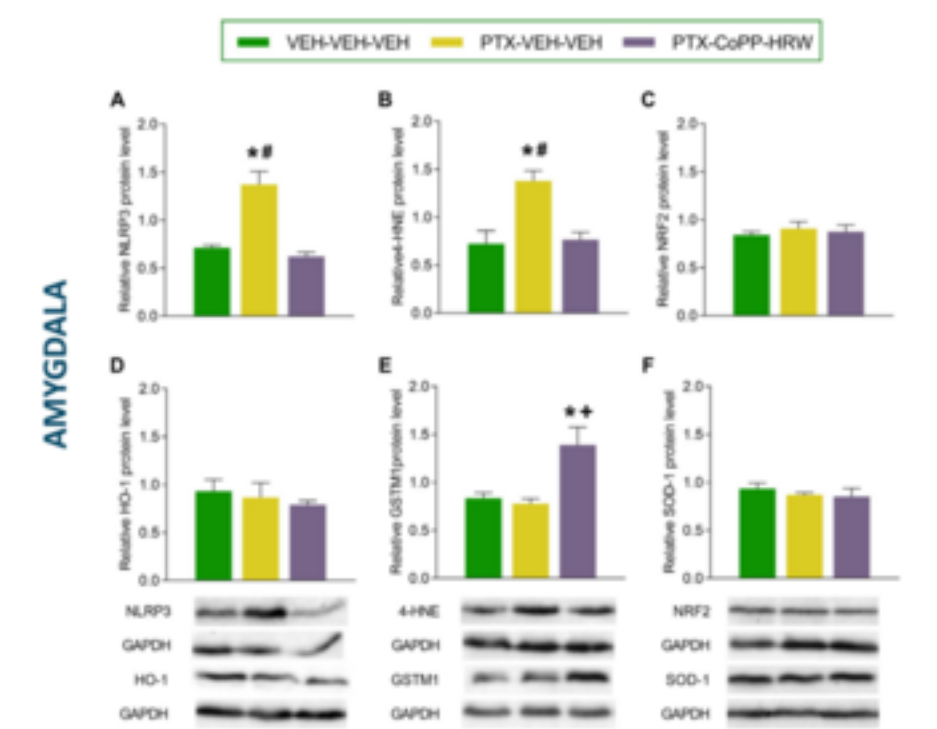


Figure 7. Effects of CoPP combined with HRW on the NLRP3, 4-HNE, NRF2, HO-1, GSTM1 and SOD-1 levels in the AMG of PTX-injected mice. This combined treatment reversed the up-regulation of NLRP3 (A) and 4-HNE (B) and increased the protein levels of GSTM1 (E) in the AMG of PTX-injected mice. No changes in NRF2 (C), HO-1 (D) and SOD-1 (F) levels were identified.



In all graphs, symbols denote significant changes vs. * VEH-VEH-VEH treated mice, + vs. PTX-injected animals treated with VEH-VEH and # vs. PTX-injected mice treated with CoPP-HRW (p < 0.05; one-way ANOVA and Tukey test). Data are presented as mean values ± SEM of 3 samples/group.

CONCLUSIONS

The results demonstrate that i) CoPP combined with HRW inhibited the neuropathy, anxiety- and depressant-like behaviors generated by PTX and ii) These effects might be a consequence of the potentiation of the antioxidant system and the inhibition of NLRP3 inflammasome activation on the DRG and/or AMG of PTX-injected mice. This study supports the importance of HO-1 and H₂ systems in modulating the PIPN and its accompanying mood disorders, and further reveals CoPP combined with HRW as a fast and effective therapy to PIPN.