ROLE OF THE HEME OXYGENASE/CARBON MONOXIDE PATHWAY IN *CLOSTRIDIUM DIFFICILE* TOXIN A-INDUCED ENTERITIS IN MICE

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INTRODUCTION

Clostridium difficile, a spore-forming and Gram-positive anaerobic bacillus, is the major cause of antibiotic-associated colitis, a disease with significant morbidity and mortality, and a major economic burden for hospitalized patients. We evaluated the role of the heme oxygenase (HO)/carbon monoxide (CO) pathway on toxin-A (TxA)-induced enteritis in mice. Numerous studies have demonstrated that HO-1, its substrate, heme and its metabolites, CO and biliverdin (BVD), are able to modulate the inflammatory process.

OBJECTIVES

To investigate the role of the hemeoxygenase (HO)/carbon monoxide (CO) pathway in *Clostridium difficile* toxin A-induced enteritis in mice.

MATERIALS AND METHODS

30 min

- **Hemin 4.5 µmol/kg (s.c)**
- **ZnPP IX 4.5 µmol/kg (s.c)**
- **DMDC 25 µmol/kg (s.c)**
- **Their respective vehicles (s.c)**

Hemin (HO substrate)

ZnPPIX (specific HO-1 inhibitor)

DMDC (CO donor)

Induction of intestinal inflammation

- **TXA** 50 µg (per ileal loop) or
- **TXA** 25 µg (per ileal loop)

3 hours

- **Sacrifice**

weight/ileal loop length ratio

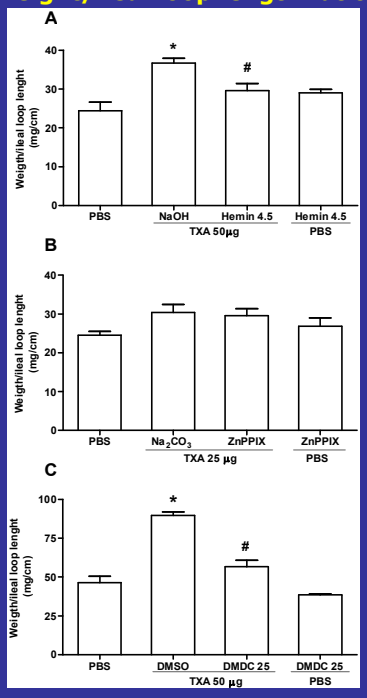
Histopathological analysis

Myeloperoxidase assay (MPO)

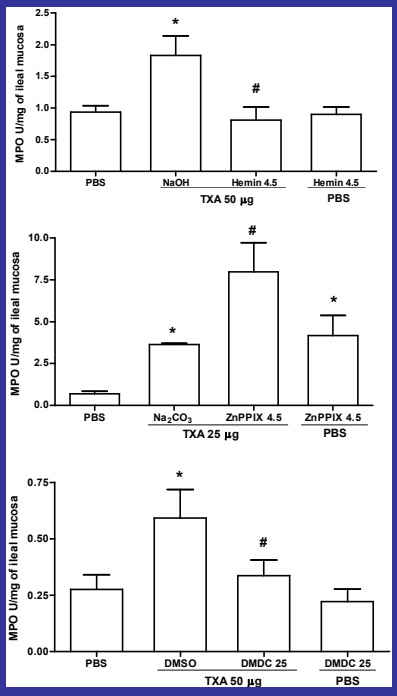
Citokines IL-1β, TNF-α by ELISA

RESULTS

Weight/ileal loop length ratio

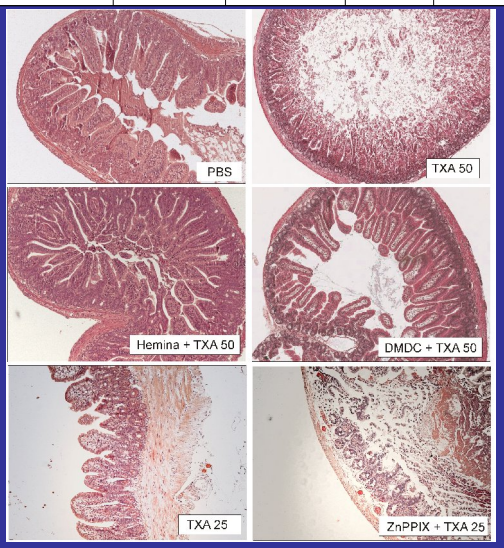


MPO activity in the ileal mucosa



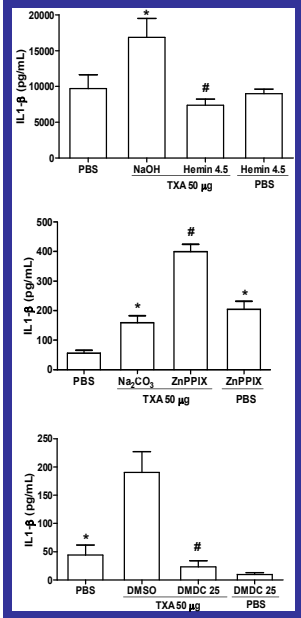
Microscopic analysis of ileal loop

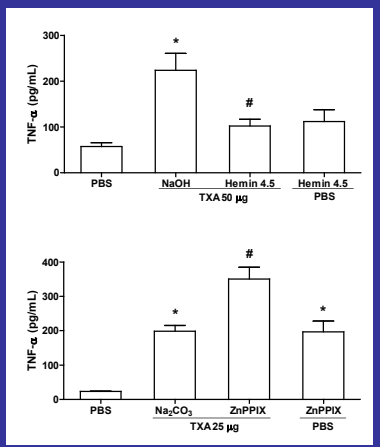
Experimental groups	Epithelial damage	Neutrophilic infiltrate	Congestion/ edema	Hemorragia
PBS	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
TXA 50 (NaOH or DMSO)	2 (2-3)*	2.5 (2-3)*	3 (2-3)*	2.5 (1-3)*
Hemin 4.5 (TXA 50)	1 (0-1) #	1 (0-1) #	1 (1-1) #	0 (0-1) #
Hemin 4.5 (PBS)	0.5 (0-1)	0 (0-0)	0.5 (0-1)	0 (0-1)
DMDC 25 (TXA 50)	1 (0-1) #	1 (0-1) #	1 (1-1) #	0 (0-1) #
DMDC 25 (PBS)	0.5 (0-1)	0 (0-0)	0.5 (0-1)	0 (0-1)
TXA 25 (Na_2CO_3)	1 (0-1)*	1 (0-1)*	0 (0-2)	0 (0-1)
ZnPPIX 4.5 (TXA 25)	2 (1-3)#	2 (1-2)#	1 (1-2)#	1.5 (0-2)#
ZnPPIX 4.5 (PBS)	0 (0-0)	1 (0-1)*	0.5 (0-1)	0 (0-0)



*P< 0.05 compared to PBS; #P< 0.05 compared to mice submitted to *Clostridium difficile* toxin A (TxA) and injected with NaOH, Na_2CO_3 or DMSO. (ANOVA) and Bonferroni's test.

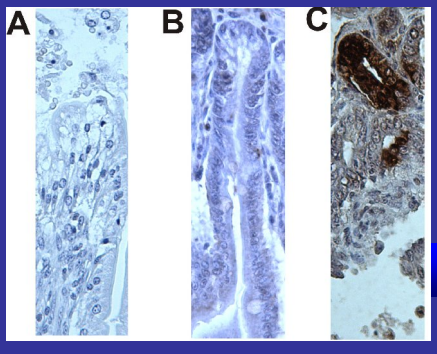
Citokines levels in the ileal mucosa of mice





*P< 0.05 compared to PBS; #P< 0.05 compared to mice submitted to *Clostridium difficile* toxin A (TxA) and injected with NaOH, Na_2CO_3 or DMSO. (ANOVA) and Bonferroni's test.

Protein HO-1 immunohistochemistry in the ileal mucosa



A – Negative control

B – PBS

C- TXA 50 µg

CONCLUSION

In conclusion, our data suggest that HO-1/CO pathway plays a protective role against *C. difficile* toxin A-induced damage.

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