Oleanolic acid protects against intestinal permeability defects and its causes in experimental autoimmune encephalomyelitis

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BACKGROUND

Multiple sclerosis (MS) is a pro-inflammatory demyelinating disease of the central nervous system, in which oxidative stress also plays an important role. Accumulating evidence from studies in patients and animal models (experimental autoimmune encephalomyelitis, EAE) suggest that disruption of intestinal homeostasis is linked to disease progression, thus representing a potential therapeutic target in MS (1). The triterpene, oleanolic acid (OA), has proven effective protecting blood-brain barrier integrity in EAE via anti-oxidant and immunomodulatory mechanisms (2), therefore, its impact on intestinal barrier homeostasis deserves investigation.

OBJECTIVES

To determine the efficacy of OA in the prevention of gut barrier alterations in EAE, with a focus on intestinal inflammatory- and oxidative-stress.

MATERIALS & METHODS

25 mg/kg/day of OA or saline were intraperitoneally administered to MOG_{35-55} immunized-C57BL/J6 mice from immunization to day 21, when untreated-EAE mice showed partial hind limb paralysis.

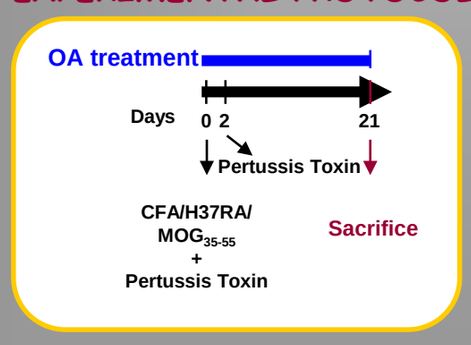
On day 21 intestinal tissue and serum samples were obtained. Analyzed parameters:

1.- Oxidative stress: lipid peroxidation (TBARS, ELISA), $O_2^{\bullet-}$ (DHE staining)

2.- Inflammation: IL-1β, TNFα (ELISA)

3.- Gut dysfunction:
- a. Markers of microbial translocation: sCD14 and iFABP (ELISA)
- b. Colon permeability (FITC-dextran) (3)
- c. Mucins (Alcian blue, AB, staining)

EXPERIMENTAL PROTOCOL



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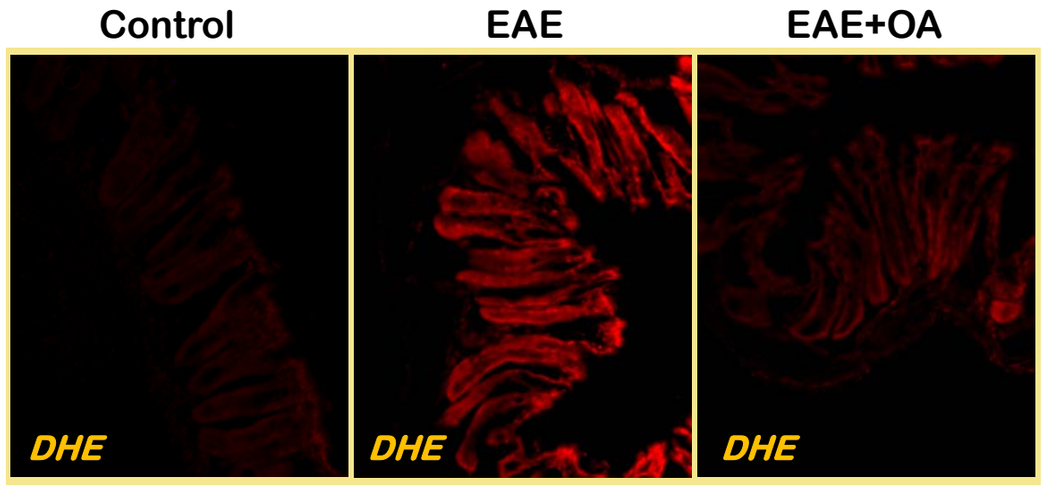
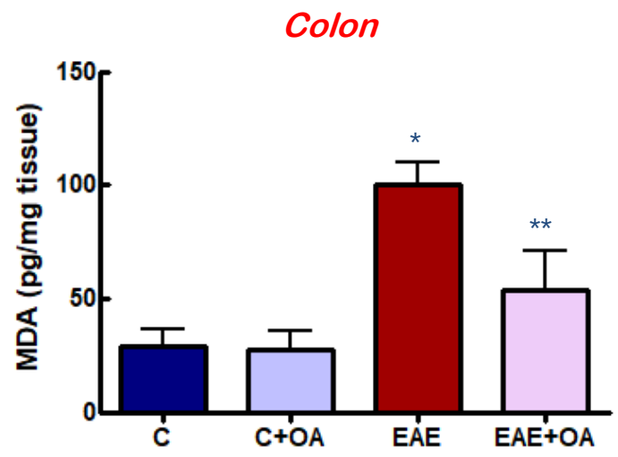
RESULTS

1.- Detection of Reactive Oxigen Species (ROS)

•A. Superoxide Anion Levels



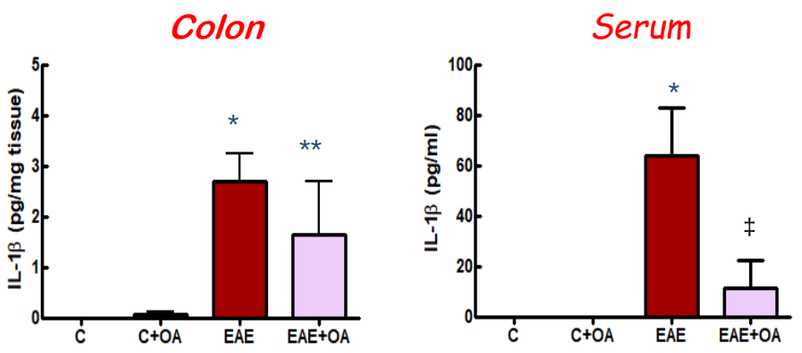
•B. Lipid Peroxidation



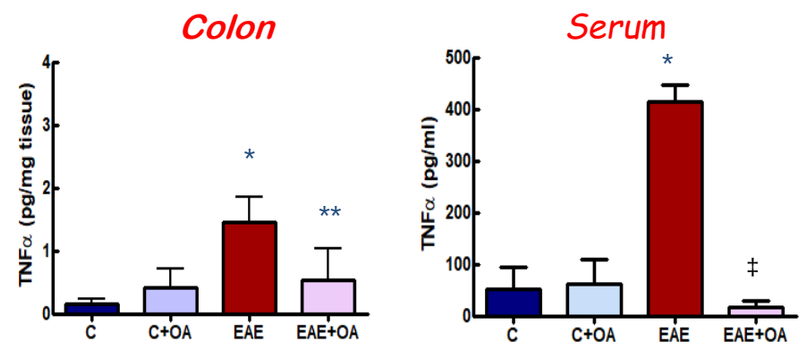
1.- OA treatment markedly reduces ROS accumulation. (A) Superoxide anion ($O_2^{\bullet-}$) and (B) Lipid peroxidation determined in colon of control, EAE and OA-treated EAE mice. (A) Representative fluorescent microscopy images of colon sections stained with dihydroethidium (DHE) to evaluate $O2^{\bullet-}$ production. (B) Malonyldialdehyde (MDA) levels were determined by a thiobarbituric acid reactive substances (TBARS) assay in colon extracts. *p<0.001 vs C; **p<0.001 vs EAE.

2.- Expression of Inflammatory Markers

A. IL-1β



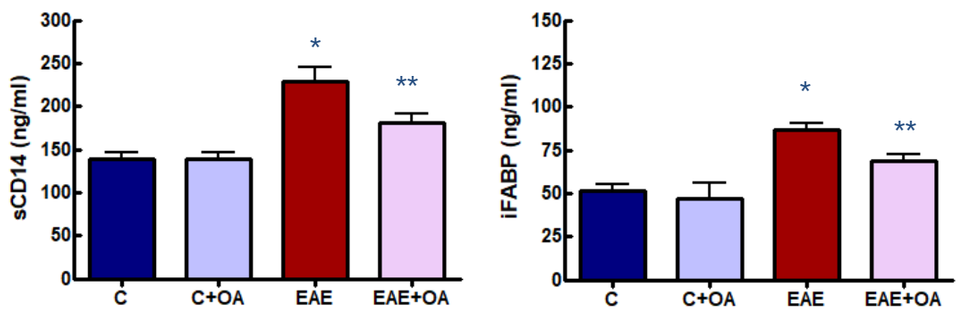
B. TNFα



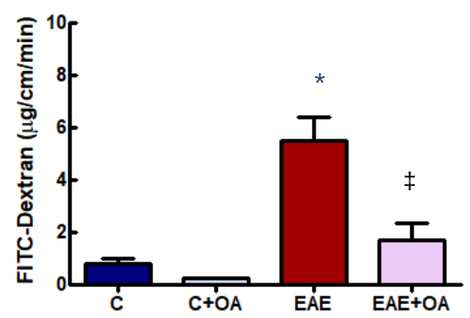
2.- OA treatment reduces the production of inflammatory proteins. The levels of IL-1β (A) and TNFα (B) were determined in serum and colon extracts of control, OA-treated control, EAE and OA-treated EAE mice by a commercial ELISA kit. *p<0.001 vs C; ‡p<0.001 vs EAE; **p<0.01 vs EAE

3.- Expression of Gut Dysfunction Markers

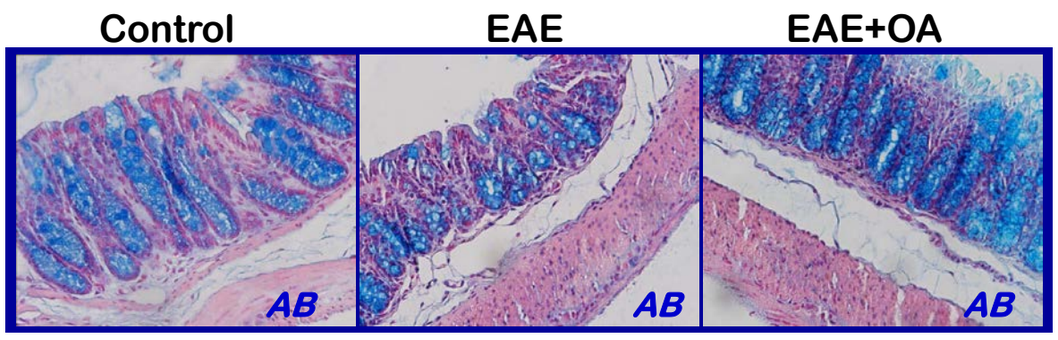
A. Markers of Microbial Translocation



B. Intestinal Permeability



C. Mucin Staining



3.- OA treatment protects against intestinal alterations. (A) Serum levels of sCD14 and iFABP were determined by a commercial ELISA kit. (B) Ex vivo intestinal permeability of FITC-dextran 40 KDa. (C) Representative microscopy images of colon sections stained with Alcian blue (AB) to evaluate mucins. *p<0.001 vs C; ‡p<0.001 vs EAE; **p<0.01 vs EAE

CONCLUSION

Our data contribute to the idea that intestinal dysfunction influences multiple sclerosis pathogenesis, and provides new findings regarding the beneficial activity of OA in EAE.

REFERENCES

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The authors have nothing to disclose