

Variability in staining specificity of commercial anti-cyclooxygenase antibodies on the neuromuscular compartment of normal human colon

Cristina Segnani¹, Chiara Ippolito¹, Rocchina Colucci², Matteo Fornai², Massimo Chiarugi³, Roberto De Giorgio⁴, Amelio Dolfi¹, Nunzia Bernardini¹

¹ Section of Histology and Medical Embriology, Department of Human Morphology and Applied Biology

² Division of Pharmacology and Chemotherapy, Department of Internal Medicine

³ Department of Surgery, Pisa University, Italy

⁴ Department of Internal Medicine and Gastroenterology, Bologna University, Italy

Background Cyclooxygenase isoforms (COX-1, COX-2) are involved in the modulation of gastrointestinal neuromuscular functions, but heterogeneous data are available on their cellular localization. Since this issue is of particular importance to understand the molecular basis of gut motility, the immunohistochemical (IHC) detection of COXs in colonic neuromuscular structures is a field of active investigation. Different staining patterns of COX tissue distribution have been reported, likely reflecting differences in gut regions, primary antibodies or detection methods.

Aim To assess the IHC staining patterns of COX-1 and COX-2 in the neuromuscular compartment of human colon by different antibodies.

Patients and Methods Full-thickness samples of macroscopically normal colon were collected from 8 patients undergone elective left hemicolectomy for colorectal neoplasia. Ten μm -thick sections from formalin-fixed, paraffin-embedded blocks were processed for IHC detection of COX-1 and COX-2 by 3 and 4 antibodies of different commercial sources, respectively.

Results and Conclusions The colonic neuromuscular structures showed considerable differences with regard for staining patterns and location of positive stains, depending on the antibody used. The immunolabelling patterns ranged from cytoplasmic to nuclear staining in ganglionic cells and muscle cells, respectively. Of note, COX-1 was mainly localized in the cytoplasm of myenteric neurons, regardless of the antibodies used. By contrast, different myenteric cell types (i.e., neurons or glial cells) were COX-2 immunolabelled, depending on the antibody. Thus, the use of different antibodies for IHC detection of COXs in human colon may yield remarkable differences in the labelling of cellular targets, leading to hardly comparable or conflicting results. These data suggest the importance of considering carefully the antibodies employed when comparing results from different IHC studies.

Key words

COX-1 and COX-2 immunohistochemistry, human colon, neuromuscular compartment