

## The wrap partial restrain stress, an animal model of the irritable bowel syndrome: immunohistochemical and functional characterization

Chiara Traini<sup>1</sup>, Stefano Evangelista<sup>2</sup>, Maria Simonetta Fausson-Pellegrini<sup>1</sup>, Maria Giuliana Vannucchi<sup>1</sup>

<sup>1</sup> Department of Experimental and Clinical Medicine. Research Unit of Histology and Embryology, University of Florence, Florence, Italy - <sup>2</sup> Preclinical Development, Menarini Ricerche SpA, Florence, Italy

Several animal models have been proposed to mimic the human irritable bowel syndrome (IBS) all based on two etio-pathogenic hypotheses: infection and stress, both responsible for the development of a local inflammation. We investigated the wrap partial restrain stress (WRS) animal model with the aim to evaluate its validity in understanding the human IBS. Male Wistar rats were used and WRS was maintained for 2h. Abdominal contractions (AC) were recorded by a distension balloon in the colon-rectum. The number of faecal pellets and their total weight were determined. Colonic specimens from both groups were examined by routine histology, immunohistochemistry and western blot (WB). WRS animals were characterized by: 1) a statistically significant increase in the number of AC and in the mean number and mean weight of faecal pellets; 2) the presence of large clusters of mononucleated cells and a significant increase in eosinophilic granulocytes and mast cells in the mucosa; 3) reduction of GLP1r-immunoreactivity (IR) located at the basolateral periphery and the Golgi level of the cells of the glandular funds; 4) an increase in CGRP-IR in the lamina propria; 5) no significant difference in the muscle wall for Cav1, L-type Ca<sup>2+</sup>-channels, Mr2, NK1r and NK2r; 6) a significant decrease in the myenteric and a significant increase in the submucous NK1-IR neuron number; 7) a significant decrease in Substance P-IR in the myenteric plexus and muscle coat; 8) a significant decrease in myenteric and submucous nNOS-IR neuron number; 9) no difference in ChAT-IR neurons of both enteric plexuses; 10) a reduction in S-100-IR in the entire colonic wall; 11) no difference in the total number of neurons evaluated by the pan-neuronal marker PGP 9.5; 12) no change of all the ICC populations. The functional data are in favor of a lowering in the colonic wall distention threshold; the morphological results obtained in the lamina propria demonstrate the presence of a local inflammation, particularly intense at the level of the mucosa. Both of these findings agree with the hypothesis that inflammation might have a main role in the insurgence and maintenance of the typical IBS symptoms and support the validity of our WRS model. Moreover, while the smooth muscle cells do not show any significant variation, numerous and consistent changes in the excitatory, inhibitory and NK1r-IR neurons are detected.

The present study was supported by Menarini Foundation Grant.