Protease activated receptor 4 expression in the intestinal tract of healthy horses

Protease activated receptor 4 (PAR4) is one of the four type of protease activated receptors’ subfamily belonging to the largest group of G-protein coupled receptors (GPCRs). PARs activation by proteases is involved in the regulation of many physiological and pathological processes in many tissues. PAR4 in humans and in animal models is extensively expressed in the gut and mainly in colon and small intestine. Immunohistochemistry from colon biopsies of humans and laboratory animals found expression of PAR4 mainly in the mucosa (especially epithelium), in the submucosa, and in cellular infiltrates. In mice PAR4 was detected also in sensory neurons projecting from the colon to the spinal ganglia (SG).

Studies in humans and in laboratory animals suggests that PAR4 is involved in the regulation of inflammation and pain mechanisms. The intracelonic administration of a PAR4 activating peptide reduce the response to colorectal distension in mice. PAR4 deficient mice showed more pain in response to colon hypersensitivity compared with control group.

Aim of the study
To evaluate, with western blot and immunohistochemistry, the distribution and expression of PAR4 in small (jejunum-JE- and ileum-IL-) and large (pelvic flexure -PF) intestines of healthy horses.

Materials and methods

Blood samples were collected at exsanguination from the jugular vein for clinicopathological evaluations. Samples of the intestines were taken at evisceration at the end of the slaughter. Those with gross and histological pathological lesions were excluded from the study.

On protein extracted from mucosa, PAR4 expression was evaluated by western blotting using primary antibody goat anti-PAR4 (1:200, sc-8464, Santa Cruz Biotechnoloy) and secondary antibody Donkey anti-Goat (1:7000, Santa Cruz sc-2020). Immunostaining was performed with sections from biopsies incubated first with primary antibody goat anti-PAR4 (1:100, sc-8464, Santa Cruz Biotechnology) and finally in secondary antibody Donkey anti-Goat (1:400, Alexa-594).

Results

Immunohistochemistry showed differences in the distribution on PAR4 in the different intestinal tracts with the higher expression in IL (Fig. 3). Staining was observed instead for cellular infiltrates that on the base of their morphology were identified as lymphocytes and mast cells (Fig. 4). These positive cellular infiltrates were highly expressed in the lamina propria of the mucosa, in the submucosa and in the sierosa (Fig. 5). PAR4 immunoreactivity was not observed in enterocytes and submucosa cells.

Conclusions

Our results supports the expression of PAR4 in the equine gut. In horses enterocytes don’t express this receptor that is instead highly express in lymphocytes and mast cells. Immunostaining of lymphocytes and mast cells for PAR4, has already been reported in humans in normal and pathological samples from colon, lung and liver. This is the first study concerning the expression and distribution of PAR4 in horses. Further study is needed to identify the expression of this receptor in equine patients referred for GI diseases. The results could clarify the role of this receptor in the modulation of visceral nociception in horses.