Bulgarian Journal of Veterinary Medicine, 2021 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2020-0127

Original article

MAST CELL DISTRIBUTION IN PORCINE COMMON BILE DUCT WITH SPECIAL REFERENCE TO GHRELIN

I. STEFANOV

Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

Stefanov, I., 2021. Mast cell distribution in porcine common bile duct with special reference to ghrelin. *Bulg. J. Vet. Med.* (online first).

Due to the similar pattern of ghrelin localisation in human and porcine intestine, swine are described as a suitable animal model for experiments related to ghrelin and its receptors. In a previous study of ours, the presence of ghrelin immunoreactive endocrine cells in common bile duct (*Ductus choledochus*) (CBD) of domestic swine was established. However, data regarding the distribution of mast cell types in the CBD in this species are missing. The current study aimed to determine the distribution of tryptase-, ghrelin- and toluidine blue positive mast cells in the wall of extra- and intramural parts of porcine CBD. For this purpose, immunohistochemical staining for detection of tryptase was performed in order to identify ghrelin reactivity in mast cells. Additionally, toluidine blue staining was carried out to compare the number of metachromatic mast cells with that of tryptase positive cells. The comparative quantitative analysis showed that the number of tryptase immunorective cells was higher than that of metachromatic ones in the CBD wall. Ghrelin immunoreactive cells were the most numerous, therefore it can be assumed that not only mast cells but other cells also contain ghrelin. The three mast cells types were more abundant in the propria and subserosa of the intramural part than in the same layers of extramural CBD part. In the muscle layer, mast cell number was higher in the extramural than in the intramural part of the duct. In conclusion, taking into account the results obtained, an important role of mast cells play in controlling CBD motility and biliary gland function was suggested.

Key words: common bile duct, domestic swine, ghrelin, mast cells, tryptase

INTRODUCTION

Mast cells (MCs) are described as immune cells that mediate allergic reactions and stimulate wound healing. They are also involved in many autoimmune, inflammatory, infectious, and other disorders (Boyce, 2004; Beaven, 2009).

The classification of mast cells is dependent on their phenotypic characteristics and anatomical locations. Reber *et al.* (2015) recently summarised the classification of mast cells in both mice and humans along with a description regarding their phenotypic characteristics. Similar to mice, human mast cells are subcategorised into tryptase-positive, and tryptase- and chymase-positive (MCTC). MCTC are

localised in the small intestinal submucosa and muscularis mucosa, whereas MCT have a tendency to inhabit the mucosa of the stomach, small intestine and colon. In rats, MCs are identified by staining for rat mast cell protease 1 (RMCP-1), which refers to connective tissue-derive mast cells, or rat mast cell protease 2 (RMCP-2), which refers to mucosal-derived MCs (Chan *et al.*, 2001; Zweifel *et al.*, 2005). The specific mast cell subtypes will determine the anatomical residency and the positioning of MCs also secures them as one of the first cells in the line of defense in the immune system.

In liver, MCs are mainly associated with the connective tissue that is found near hepatic arteries, veins and bile ducts of the portal tracts in both human and rat livers. In normal rodent and human livers, MCs are found in small numbers along the portal tracts (Kennedy *et al.*, 2014; Johnson *et al.*, 2016; Jones *et al.*, 2016). However, their density increases during different hepatic injuries such hepatocellular carcinoma (Terada & Matsunaga, 2000), cholangiocarcinoma (Terada and Matsunaga, 2000; Johnson *et al.*, 2016), primary sclerosing cholangitis (PSC) (Baron *et al.*, 1995; Tsuneyama *et al.*, 2000); human and rodent bile duct obstruction (Gulubova & Vlaykova, 2004). Mast cell localisation in close proximity to bile ducts during various cholangiopathies indicates that MCs and cholangiocytes may regulate one another via paracrine signalling. Mast cells interact with various liver cell types (macrophages, or Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells, vascular endothelial cells, fibroblasts and pit cells) having various functions in normal liver and in hepatic diseases. It has been ascertained that all these cells (in addition to biliary cells) play definitive roles in liver pathophysiology and determine complex interactions with hepatic MCs (Grizzi *et al.*, 2013). According to Tsuneyama *et al.* (1999), MCs expressed bFGF and/or TNF-α, which are well known promoters of fibrosis. In PSC samples, the aberrant expression of SCF was found on biliary epithelia of dilated and stenotic bile ducts that displayed periductal fibrosis and inflammation, while there was no expression in non-affected bile ducts in normal livers (Tsuneyama *et al.*, 1999). It may be conceivable that the aberrantly expressed SCF found on biliary epithelial cells accumulates and attracts/stimulates mast cells via the c-Kit receptor, and these activated mast cells induce progressive periductal and portal fibrosis during PSC. Johnson *et al.* (2016) reported that mast cells are involved in the progression of cholangiocarcinoma via increased angiogenesis and metastatic potential.

Ghrelin (Gr) is a 28 amino acid peptide that was established for the first time in rat stomach (Kojima *et al.*, 1999). It is the natural ligand for the growth hormone secretagogue receptor (GHS-R) (Howard *et al.*, 1996). The seven-transmembrane GHS-R has a high degree of homology ranging from 93% to 99% identity by molecular analysis with those of humans, pigs, dogs, rats and mice (Howard *et al.*, 1996; Smith *et al.*, 1996; McKee *et al.*, 1997; Dieguez & Casanueva, 2000; Dong *et al.*, 2009). Porcine GHS-R also has two forms: GHSR-1a consists of 366 amino acid peptides, and GHSR-1b encodes 289 amino acids (Dong *et al.*, 2009).

Wierup *et al.* (2007) found the pig to be a suitable animal model for secretion studies because the pattern of colocalisation of Gr and motilin in the porcine intestine (duodenum and jejunum) was very similar to that of the human intestine. These authors reported that the two hor-

mones are costored in the same granules and suggested that the two hormones are co-secreted from the same cells.

Ghrelin has been reported to be predominantly expressed in the digestive system, with highest expression levels in the gastric mucosa (Kojima *et al.*, 1999; Ariyasu *et al.*, 2001). Other authors detected Gr expression also in other tissues such as kidneys, adrenal glands, thyroid gland, breast, ovary, placenta, testis, prostate, liver, gallbladder, lung, skeletal muscles, myocardium, skin, and bone (Gnanapavan, *et al.*, 2002; Ghelardoni *et al.*, 2006).

Later, Gulubova *et al.* (2017) described the peptide localisation in endocrine cells and nerve structures of porcine common bile duct. However, data regarding the distribution of mast cell types in the common bile duct in this species are absent.

Based on a previous study of ours (Stefanov *et al.*, 2017) where ghrelin positive mast cells (MCGr+) were identified for the first time in rat stomach, this study was performed to define the expression of this peptide by mast cells and their distribution in porcine common bile duct.

MATERIALS AND METHODS

Tissue preparation

For this study, the terminal segment of the extramural part as well as the intramural part of CBD of 6 male pigs at the age of 6 months were immediately removed after slaughtering in a slaughterhouse. The tissue specimens were put in 10% aqueous formalin solution for 24 hours, washed with PBS, dehydrated in an alcohol, cleared in xylene, and embedded in paraffin. The activities were performed within Scientific Project 13/2017 of Medical Faculty of Trakia University, Stara Zagora, Bulgaria.

Serial tissue sections of 5 μm thickness from each animal were cut, mounted on gelatin coated slides, deparaffinised in xylene and rehydrated by a series of decreasing ethanol concentrations. The tissue sections were processed by immunohistochemical reactions to detect tryptase and ghrelin reactivity as well as histochemical reaction using toluidine blue dye in order to define metachromatic cells.

Toluidine blue staining for metachromasia visualisation

Tissue sections were mounted on gelatinised slides, placed in xylene, rehydrated by decreasing ethanol concentrations and stained in a buffered solution of toluidine blue (pH = 3).

Immunohistochemical staining for detection of tryptase and ghrelin expression

The tissue sections were washed in 0.1 M PBS and placed in 1.2% hydrogen peroxide in methanol for 30 minutes. Antigen recovery in buffer (pH 9.0) was performed, then incubated in a humidified chamber overnight at 4 °C with primary antibodies: mouse antihuman ghrelin (2F4) at 1:50 dilution, monoclonal mouse antihuman mast cell tryptase – ready for use. After washing with PBS, the sections were incubated with EnVision detection system (DAKO) for 24 hours at 4 °C. The immune reaction was visualised with diaminobenzidine. PBS instead of the primary antibody was used as a negative control. The slices were dehydrated, washed, coated with coverslips and photographed with a research microscope (LEIKA DM1000) equipped with a digital camera (LEIKA DFC 290). Of the three serial sections used, one was stained with tryptase, another with ghrelin and the last – with toluidine blue for metachromasia.

Statistical analysis

Mast cells density (number/field of view) was estimated on the microscopic fields ×200 with an area of 0.163 mm^2 using a light research microscope (LEICA DM1000) with a digital camera (LEICA DFC 290). Mast cell density data were processed using GraphPadPrism 6 for Windows (GraphPad Software, Inc., USA) for analysis of variations (one-way ANOVA), followed by Tukey-Kramer test. All data were expressed as means ± standard deviation (SD) with a p-value less than 0.05 set as level of significance.

RESULTS

Tryptase and ghrelin expressions in MCs were defined by immunohistochemical reactions but metachromatic mast cells (MCtb+, showing γ-metachromasia) were manifested using toluidine blue staining (Fig. 1–3). Co-localisation with tryptase on serial sections showed that most of the ghrelin positive cells (Cgr+) are also tryptase positive MCs (MCTr+).

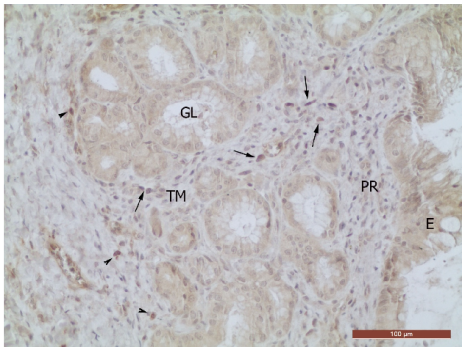

Fig. 1. Some of the ghrelin immunoreactive cells are tryptase positive (arrows), but others are tryptase negative (arrowheads). PR – propria, TM –*Tunica muscularis*, E – *Lamina epithelialis mucosae*, GL – *Glandulae ductus choledochi*, c – capillary. Bar=100 µm.

In the propria, tryptase-, ghrelin positive MCs and metachromatic MCs were localised mainly near the capillaries, arterioles and venules. Mast cells also were observed near the biliary glands (Fig. 1–3). The statistical analysis showed that in all layers of the extra- and intramural part of the CBD, the number of Cgr+ was the highest, followed by those of MCtr+ and MCtb+ (Table 1, Fig. 4–6). Therefore, some of the Cgr+ are MCghr+ but other Cgr+ were not mast cells.

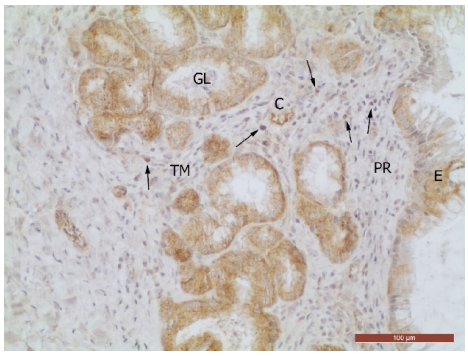

Fig. 2. Tryptase positive mast cells in the propria (PR) and *Tunica muscularis* (TM). E – *Lamina epithelialis mucosae*, GL – *Glandulae ductus choledochi*, c – capillary. Bar=100 µm.

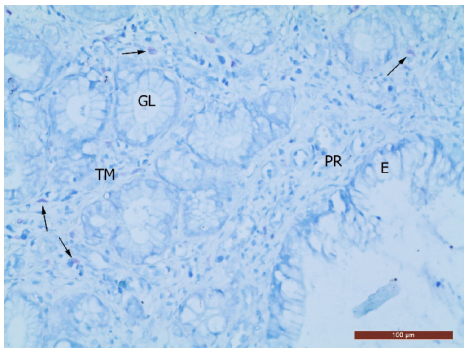

Fig. 3. Mast cells with metachromasia in the propria (PR) and *Tunica muscularis* (TM). E – *Lamina epithelialis mucosae*, GL – *Glandulae ductus choledochi*. Bar=100 µm.

Table 1. Number (mean±SD) of tryptase (MCtr+) - and toluidine blue (MCtb+) positive mast cells as well as of ghrelin positive cells (Cgr+) in the layers of the extramural (DCHT) and intramural part (DCHI) of porcine common bile duct

Mast cell localisation	MCtr+ number	Cgr+ number	MCtb+ number
• DCHT	*A4/D4*	*A4/D4*	*A4/D4*
Lamina propria mucosae	8.22 ± 0.73	10.50 ± 0.51	2.89 ± 0.76
• DCHT	*A4/C4*	*A4/C4*	*A4/C4*
Tunica muscularis	17.83 ± 1.58	21.61 ± 1.42	15.94 ± 0.73
• DCHT	*A4/B4*	*A4/B4*	*A4/B4*
Tela subserosa	9.94 ± 0.87	12.89 ± 0.76	7.50 ± 0.71
• DCHI			
Lamina propria mucosae	*A4/D4*	*A4/D*4	*A4/D4*
SECTL	22.61 ± 1.24	28.78 ± 1.17	1.55 ± 0.51
	F4	*A4/F4*	*A4/F4*
SGCTL	16.33 ± 0.69	19.17 ± 0.79	16.17 ± 0.79
• DCHI			
Tunica muscularis	*C4*	*A4/C*4	*A4/C4*
circular muscle layer	12.50 ± 0.92	16.39 ± 1.38	12.83 ± 0.86
	A2/E4	*A2/E4*	*A4/E4*
longitudinal muscle layer	10.33 ± 0.69	11.83 ± 1.78	9.00 ± 0.84
• DCHI	*A4/B4*	*A4/B4*	*A4/B4*
Tela subserosa	7.78 ± 1.0	18.44 ± 1.04	5.83 ± 0.78

SECTL – subepithelial connective tissue layer, SGCTL –subglandular connective tissue layer. Statistically significant differences: *A2* (P<0.01), *A4* (P<0.0001) – between MCtr+, Cgr+ and MCtb+ numbers at the same layer; *B4* (P<0.0001) – in MCtr+, Cgr+ and MCtb+ number between the serous and muscle layer; *C4* (P<0.0001) – in MCtr+, Cgr+ and MCtb+ number between the muscle layer and propria; *D4* (P<0.0001) – in MCtr+, Cgr+ and MCtb+ number between the propria and serous layer; *E4* (P<0.0001) – in MCtr+, Cgr+ and MCtb+ number between the circular and longitudinal muscle layers of *Tunica muscularis; F4* (P<0.0001) – in MCtr+, Cgr+ and MCtb+ number between the SECTL and SGCTL of the propria.

The comparative study showed that out of all Cgr+ in the propria of the terminal segment of the extramural part of common bile duct (DCHT), 78% were MCTr+ and 28% were MCtb+. Out of all MCTr+, 35% were MCtb+.

Lamina propria mucosae of the intramural part of common bile duct (DCHI) consisted of subepithelial and subglandular connective tissue layers. In DCHI subepithelial connective tissue layer, out of all Cgr+, 79% were MCTr+ and 5% were MCtb+. Seven percents of all MCTr+, were MCtb+. In DCHI subglandular connective tissue layer, out of all Cgr+, 85% were MCTr+ and 84% were MCtb+. The number of MCTr+ was equal to that of MCtb+.

In the *tunica muscularis*, the three types of MCs were situated near the smooth muscle cells, blood vessels and biliary glands. Similarly to the propria, the number of Cgr+ was the highest, followed by MCtr + and MCtb+.

In the *tunica muscularis* (fibromuscular layer) of DCHT, from all, 83% of

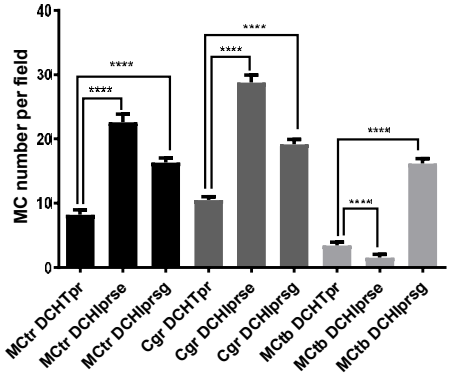

Fig. 4. Comparative quantitative analysis between tryptase (MCtr), toluidine blue (MCtb) positive mast cells and ghrelin positive (Cgr) cells in the propria of the extramural part and those in the propria (sibepithelial and subglandular layers) of the intramural part of common bile duct. The number of MCtr and Cgr in the propria of extramural part of the common bile duct (DCHTpr) and the subepithelial connective tissue layer of the intramural part of common bile duct (DCHIse) was significantly higher than in the subglandular connective tissue layer of common bile duct (DCHIsg) in 6 month-old pigs. However, the number of MCtb is the highest in DCHIsg, followed by that in DCHTpr and in DCHIse. **** P<0.0001.

Cgr+ were MCTr+ and 74% were MCtb+. Out of all MCTr+, 89% were MCtb+. In the circular muscle layer of DCHI *tunica muscularis*, 76% of all Cgr+ were MCTr+ and 78% were MCtb+. The number of MCTr+ was equal to that of MCtb+. In the DCHI longitudinal muscle layer of *tunica muscularis*, 87% of all Cgr+ were MCTr+ and 76% – MCtb+. Out of all MCTr+, 87% were MCtb+.

In the *tela subserosa*, MCs were detected close to the vessels and nerves. Like in the propria and muscle layer, Cgr+ here were also the most numerous, followed by MCtr+ and MCtb+. In the subserous layer of DCHT, out of all Cgr+, 77% were MCTr+ and 58% were MCtb+. Out of all MCTr+, 76% were MCtb+. In the subserous layer of DCHI, 42% of Cgr+ were MCTr+ and 32% were MCtb+. Out of all MCTr+, 75% were MCtb+.

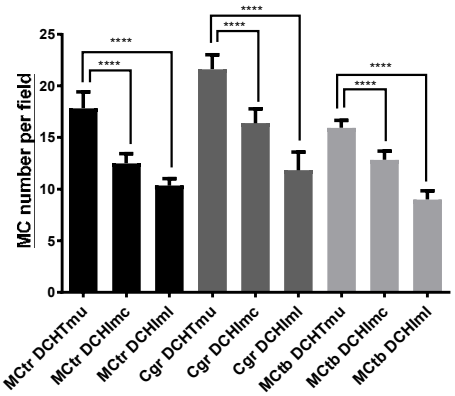

Fig. 5. Comparative quantitative analysis between tryptase (MCtr), toluidine blue (MCtb) positive mast cells and ghrelin positive (Cgr) cells in *Tunica muscularis* of extramural part (DCHTmu) and those in *Tunica muscularis* - circular (DCHTmc) and longitudinal muscle layers (DCHTml), of the intramural part of the common bile duct (DCHI) in 6 month-old pigs. The number of MCtr, MCtb and Cgr is the highest in the muscle layer of DCHT, followed by those in circular and longitudinal muscle layer of DCHI. **** P<0.0001.

DISCUSSION

This study was the first to supply information about the distribution of MCtr+ , MCtb+ ans MCgr+ in the three main layers of the common bile duct's wall.

So far, some authors (Gulubova & Vodenicharov, 2001; Gulubova & Vlaykova, 2004) have provided information about the distribution of tryptase-, chymase-, vasointestinal polypeptide (VIP)-, and substance P (SP)-positive MCs in

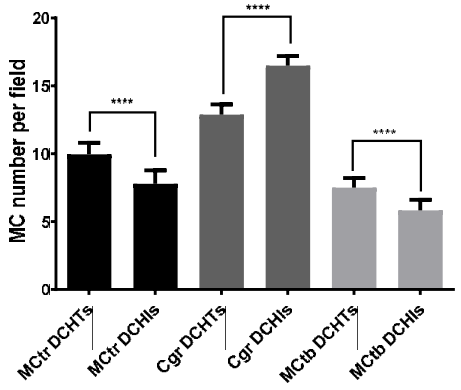

Fig. 6. Comparative quantitative analysis between tryptase (MCtr), toluidine blue (MCtb) positive mast cells and ghrelin positive (Cgr) cells in subserosal layer of extramural part (DCHTs) and those in subserosal layer of intramural part (DCHIs) of common bile duct in pigs at the age of 6 months. The number of MCtr and MCtb in DCHTs is higher than that in DCHIs, whereas the number of Cgr in DCHIs: higher than that in DCHTs; **** P<0.0001.

human normal CBD and in pathologic conditions. Jones *et al.* (2016) examined the effects of mast cells and mast cell-derived histamine on bile flow, total bile acid pool and bicarbonate excretion – the first study showing that mast cell-derived histamine may regulate biliary proliferation and hepatic fibrosis in human PSC. In the human common bile duct with biliary obstruction, increased numbers of mast cells were detected (Gulubova & Vodenicharov, 2001; Gulubova & Vlaykova, 2004). Later, Gulubova *et al.* (2017) described the peptide localisation in endocrine cells and nerve structures of porcine common bile duct. However, data regarding the distribution of mast cell types in the porcine common bile duct in this species are absent. Terada *et al.* (2000) noted that there were significantly more mast cells in human intrahepatic cholangiocarcinoma tumours when compared to normal livers, and that 20% of the mast cells were MCtr+ while the other 80% were MCTC. These findings highlighted the significant role of mast cells during tumourigenesis, and assisted in identification of the specific mast cell subsets, helping future researchers in developing a potential therapeutic target.

In the current study, co-localisation with tryptase on serial sections showed that most of the Cgr+ were MCTr+, and helped defining MCgr+. For example, in the propria of DCHT, MCgr+ were 78% from all Cgr+, while in the subepithelial and subglandular connective tissue layer of DCHI, MCgr+ were 79% and 85%, respectively. These results are supported by a previous study of ours (Stefanov *et al.*, 2017) where ghrelin expression by mast cells in rat stomach was detected.

Our findings in the propria showed that MCtr+, MCgr+ and metachromatic MCs were localised predominantly near the capillaries, arterioles and venules. This localisation is probably related to the role of MCs in regulating blood vessel relaxation and permeability (McCauley *et al.*, 2005). Mast cells were also observed near the biliary glands where they may regulate glandular secretion in line with the results of Penkova *et al.* (2016) who studied the ghrelin expression in human stomach and intestine. By identifying MCtr+ and MCgr+, we added to existing data about the mast cell phenotypes in the porcine biliary tract. The highest number of Cgr+ compared to the number of MCtr+ and MCtb+ indicated that some Cgr+ were MCghr+ but other Cgr+ that were different from MC, were probably lymphocytes (B cells), T cells, monocytes and NK cells (Dixit *et al.*, 2004; Hattori, 2009; Taub *et al.*, 2010).

In the *tunica muscularis* of extramural part of CBD, the three MCs types were situated near the smooth muscle cells, blood vessels and biliary glands but in the *tunica muscularis* of intramural intramural part of CBD the three types of MCs were situated near the smooth muscle cells and blood vessels. The number of Cgr+ was the highest. In the muscle layer of DCHT, MCgr+ were 74% of all Cgr+, while in the circular and longitudinal muscle layers of *tunica muscularis* in DCHI – 78% and 87%, respectively. It is acknowledged that gastric motility is stimulated by ghrelin inducing the migrating motor complex and accelerating gastric emptying (Dass *et al.*, 2003; Fujino *et al.*, 2003; Depoortere *et al.,* 2005; Peeters, 2005; 2013). Taking into account the role of ghrelin in controlling gastric emptying (Hilsted *et al.*, 2005) and intestinal motility (Edholm *et al.*, 2004; Penkova *et al,,* 2016) we suggest that this peptide may be considered a main regulator of the motility of common bile duct.

In the *tela subserosa*, MCs were detected close to the vessels and nerves. In the subserous layer of DCHT, MCgr+ were 77% of all Cgr+, while in the same layer of DCHI, MCgr+ comprised 42% of all Cgr+. This is evidence that mast cells and nerves were probably in bidirectional communication correlating with previous investigations (Dimitriadou *et al.*, 1987; Barbara *et al.,* 2006; Stefanov & Vodenicharov, 2016). Substance P (SP) and nerve growth factor (NGF) released from peripheral sensory nerves cause mast cell degranulation. Histamine, produced and secreted by the mast cells, activates peripheral sensory nerves (Theodorou, 1996). Chen *et al.* (1998) reported that sympathetic neurotransmission caused decreased gallbladder tone and contributed to its filling.

CONCLUSION

Ghrelin containing mast cells with highest number in the muscle layer together with the mast cell localisation near the biliary glands defined the important role of these cells in regulation of the motility of porcine common bile duct and of biliary gland secretion. In the studied structures, the higher density of MCTr+ vs MCtb+, defined tryptase as a better marker for MCs than the toluidine blue dye.

REFERENCES

Ariyasu, H., K. Takaya, T. Tagami, Y. Ogawa, K. Hosoda, T. Akamizu, M. Suda, T. Koh, K. Natsui, S. Toyooka, G. Shirakami, T. Usui, A. Shimatsu, K. Doi, H. Hosoda, M. Kojima, K. Kangawa & K. Nakao, 2001. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4753–4758.

Barbara, G., R. De Giorgio, V. Stanghellini & R. Corinaldesi, 2006. Functional gastrointesinal disorders and mast cells; implications for therapy. *Neurogastroenterology and Motility*, **18**, 6–17.

Baron, T. H., R. E. Koehler, W. H. Rodgers, M. B. Fallon & S. M. Ferguson, 1995. Mast cell cholangiopathy: another cause of sclerosing cholangitis. *Gastroenterology*, **109**, 1677–1681.

Beaven, M. A., 2009. Our perception of the mast cell from Paul Ehrlich to now. *European Journal of Immunology*, **39**, 11–25.

Boyce, J. A., 2004. The biology of the mast cell. *Allergy and Asthma Proceedings*, **25**, 27–30.

Chan, A., M. A. Cooley & A. M. Collins, 2001. Mast cells in the rat liver are phenotypically heterogeneous and exhibit features of immaturity. *Immunology and Cell Biology*, **79**, 35–40.

Chen, Q., K. Lee, Z. Xiao, P. Biancani & J. Behar, 1998. Mechanism of gallbladder relaxation in the cat: Role of norepinephrine. *Journal of Pharmacology and Experimental Therapeutics,* **285,** 475–479.

Dass, N. B., M. Munonyara, A. K. Bassil, G. J. Hervieu, S. Osbourne, S. Corcoran, M. Morgan & G. J. Sanger, 2003. Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin. *Neuroscience*, **120**, 443–453.

Depoortere, I., B. De Winter, T. Thijs, J. De Man, P. Pelckmans & T. Peeters, 2005. Comparison of the gastroprokinetic effects of ghrelin, GHRP-6 and motilin in rats *in vivo* and *in vitro*. *European Journal of Pharmacology*, **515**, 160–168.

Dieguez, C. & F. F. Casanueva, 2000. Ghrelin: A step forward in the understanding of somatotroph cell function and growth regulation. *European Journal of Endocrinology*, **142**, 413–417.

Dimitriadou, V., P. Aubineau, J. Taxi & J. Seylaz, 1987. Ultrastructural evidence for a functional unit between nerve fibers and type II cerebral mast cells in the cerebral vascular wall. *Neuroscience*, **22**, 621–630.

Dixit, V. D., E. M. Schaffer & R. S. Pyle, 2004. Ghrelin inhibits leptin and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *Journal of Clinical Investigation*, **114**, 57–66.

Dong. X-Y., J. Xu, Sh.-Q. Tang, H.-Y. Li, Q.-Y. Jiang & X.-T. Zou, 2009. Ghrelin and its biological effects on pigs. *Peptides*, **30**, 1203–1211.

Edholm, T., F. Levin, P. M. Hellström & P.T. Schmidt, 2004. Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. *Regulatory Peptides*, **121**, 25–30.

Fujino, K., A. Inui, A. Asakawa, N. Kihara, M.Fujimura & M. Fujimiya, 2003. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *Journal of Physiology*, **550**, 227–240.

Ghelardoni, S., V. Carnicelli, S. Frascarelli, S. Ronca-Testoni & R. Zucchi, 2006. Ghrelin tissue distribution: Comparison between gene and protein expression. *Journal of Endocrinological Investigation,* **29**, 115–121.

Gnanapavan, S., B. Kola, S. A. Bustin, D. G. Morris, P. McGee, P. Fairclough, S. Bhattacharya, R. Carpenter, A. B. Grossman & M. Korbonits, 2002. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *Journal of Clinical Endocrinology and Metabolism*, **87**, 2988–2991.

Grizzi, F., G. Di Caro, L. Laghi, P. Hermonat, P. Mazzola, D. D. Nguyen, S. Radhi, J. A. Figueroa, E. Cobos, G. Annoni & C. Internati, 2013. Mast cells and the liver aging process. *Immunity and Ageing*, **10**, 9.

Gulubova M. V. & T. I. Vlaykova, 2004. Mast cells in human bile duct obstruction. *Journal of Molecular Histology,* **35**, 791–801.

Gulubova, M. V., I. V. Valkova, K. V. Ivanova, I. G. Ganeva, D. K. Prangova, M. M. K. Ignatova, S. R. Vasilev & I. S. Stefanov, 2017. Endocrine cells in pig's gallbladder, ductus cysticus and ductus choledochus with special reference to ghrelin. *Bulgarian Chemical Communications,* Special Issue E, 184–190.

Gulubova, M. & A. Vodenicharov, 2001. Structural examination of tryptase-, and VIP-positive mast cells in the common bile duct of patients with lithiasis. *Acta Histochemica*, **103,** 437–452.

Hattori, N., 2009. Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. *Growth Hormone and IGF Research*, **19**, 187–197.

Hilsted, L. M., P. M. Hellström & E. Naslund, 2005. Effect of peripherally administered ghrelin on gastric emptying and acid secretion in the rat. *Regulatory Peptides,* **131**, 59–65.

Howard, A. D., S. D. Feighner, D. F. Cully, J. P. Arena, P. A. Liberator, C. I. Rosenblum, M. Hamelin, D. L. Hreniuk, O. C.

Palyha, J. Anderson, P. S. Paress, C. Diaz, M. Chou, K. K. Liu, K. K. McKee, S. S. Pong, L. Y. Chaung, A. Elbrecht, M. Dashkevicz, R. Heavens, M. Rigby, D. J. Sirinathsinghji, D. C. Dean, D. G. Melillo, A. A. Patchett, R. Nargund, P. R. Griffin, J. A. DeMartino, S. K. Gupta, J. M. Schaeffer, R. G. Smith & L. H. Van der Ploeg, 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science,* **273**, 974–977.

Johnson, C., V. Huynh, L. Hargrove, L. Kennedy, A. Graf-Eaton, J. Owens, J.P. Trzeciakowski, K. Hodges, S. DeMorrow, Y. Han, L. Wong, G. Alpini & H. Francis, 2016. Inhibition of mast cell-derived histamine decreases human cholangiocarcinoma growth and differentiation via c-Kit/stem cell factor-dependent signaling. *American Journal of Pathology*, **186**, 123–133.

Jones, H., L. Hargrove, L. Kennedy, F. Meng, A. Graf-Eaton, J. Owens, G. Alpini, C. Johnson, F. Bernuzzi, J. Demieville, S. DeMorrow, P. Invernizzi & H. Francis, 2016. Inhibition of mast cell-secreted histamine decreases biliary proliferation and fibrosis in primary sclerosing cholangitis Mdr2-/- mice. *Hepatology*, **64**, 1202–1216.

Kennedy, L. L., L. A. Hargrove, A. B. Graf, T. C. Francis, K. M. Hodges, Q. P. Nguyen, Y. Ueno, J. F. Greene, F. Meng, V. D. Huynh & H. L. Francis, 2014. Inhibition of mast cell-derived histamine secretion by cromolyn sodium treatment decreases biliary hyperplasia in cholestatic rodents. *Laboratory Investigation*, **94**, 1406–1418.

Kojima, M., H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, & K. Kangawa, 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, **402**, 656–660.

McCauley, S., M. Gilchrist & A. D. Befus, 2005. Nitric oxide: A major determinant of mast cell phenotype and function. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro*, **100**, 11–14.

McKee, K. K., O. C. Palyha, S. D. Feighner, D. L. Hreniuk, C. P. Tan, M. S. Phillips, R. G. Smith, L. H. Van der Ploeg & A. D. Howard, 1997. Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. *Molecular Endocrinology*, **11**, 415–423.

Peeters, T. L., 2005. Ghrelin: A new player in the control of gastrointestinal functions. *Gut*, **54**, 1638–1649.

Peeters, T. L., 2013. Ghrelin and the gut. *Endocrine Development*, **25**, 41–48.

Penkova, N., P. Atanasova, Z. Zheleva & M. Chilikova, 2016. Ghrelin and its role in the secretion and motility of the gastrointestinal tract in humans. *IOSR Journal of Dental and Medical Sciences*, **15**, 107–113.

Reber, L. L., R. Sibilano, K. Mukai & S. J. Galli, 2015. Potential effector and immunoregulatory functions of mast cells in mucosal immunity. *Mucosal Immunology*, **8**, 444–463.

Stefanov, I., J. Ananiev, K. Ivanova, A. Tolekova, A. Vodenicharov & M. Gulubova, 2017. Distribution of ghrelin positive mast cells in rat stomach. *Biotechnology and Biotechnological Equipment,* **31**, 774–781.

Smith, R. G., S. S. Pong, G. Hickey, T. Jacks, K. Cheng, R. Leonard, C. J. Cohen, J. P. Arena, C. H. Chang, J. Drisko, M. Wyvratt, M. Fisher, R. Nargund & A. Patchett, 1996. Modulation of pulsatile GH release through a novel receptor in hypothalamus and pituitary gland. *Recent Progress in Hormone Research*, **51**, 261–285.

Taub, D. D., W. J. Murphy & D. L. Longo, 2010. Rejuvenation of the aging thymus: growth hormone-mediated and ghrelin mediated signaling pathways. *Current Opinion in Pharmacology*, **10**, 408–424.

Terada, Y. & T. Y. Matsunaga, 2000. Increased mast cells in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *Journal of Hepatology*, **33**, 961–966.

Theodorou, V., J. Fioramonti & L. Bueno, 1996. Integrative neuroimmunology of the

digestive tract. *Veterinary Research*, **27**, 427–442.

Tsuneyama, K., N. Kono, M. Yamashiro, W. Kouda, Sabit A, M. Sasaki & Y. Nakanuma, 1999. Aberrant expression of stem cell factor on biliary epithelial cells and peribiliary of c-kit-expressing mast cells in hepatolithiasis and primary sclerosing cholangitis: a possible contribution to bile duct fibrosis. *Journal of Pathology*, **189**, 609–614.

Wierup, N., M. Björkqvist, B. Weström, S. Pierzynowski, F. Sundler & K. Sjölund, 2007. Ghrelin and motilin are cosecreted from a prominent endocrine cell population in the small intestine. *Journal of Clinical Endocrinology and Metabolism*, **92**, 3573–3581.

Zweifel, M., K. Breu, K. Matozan, E. Renner, M. Welle, T. Schaffner & P. A. Clavien, 2005. Restoration of hepatic mast cells and expression of a different mast cell protease phenotype in regenerating rat liver after 70%-hepatectomy. *Immunology and Cell Biology*, **83**, 587–595.

Paper received 16.07.2020; accepted for publication 01.12.2020

Correspondence:

Assoc. Prof. Ivaylo Stefanov
Department of Anatomy, Faculty of Medicine,
Morphoblock, 11 Armeiska Street
Trakia University,
6000 Stara Zagora, Bulgaria
e-mail: ivstefanov@abv.bg