Mast cells degranulation triggers intra-abdominal adhesion after laparoscopic

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Running Head: Laparoscopic effect on mast cell degranulation and intra-abdominal adhesion

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Abstract

Background: Laparoscopic at specific pressures has potential intra-abdominal adhesion. Unfortunately, the pathomechanism of intra-abdominal adhesion is still challenging to understand. Proving the effect of mast cell degranulation with intra-abdominal adhesion was the aim of this study.

Methods: Thirty male Sprague-Dawley rats were grouped into five groups (n = 6 per group), namely: a) the control group and b) the intervention group 5 mmHg, 8 mmHg, 10 mmHg, and 12 mmHg performed 60 minutes insufflation using carbon dioxide (CO_2) at 5, 8, 10 and 12 mmHg, respectively. Seven days after laparoscopy, our study evaluated: a) the number and percentage of mast cell degranulation in the peritoneum, mesentery, and omentum; b) histamine, tryptase, and chymase of peritoneal fluid; c) thickness of extracellular matrix peritoneal tissue and d) intra-abdominal adhesion scoring.

Results: There was a statistically significant higher in a) mast cell infiltration and degranulation, b) histamine and tryptase levels of peritoneal fluid, c) extracellular matrix thickness, and d) adhesion scoring at 10 mm Hg ($p < 0.05$).

Conclusions: Our study proved that laparoscopy results in mast cell degranulation that increases in intra-abdominal adhesion.

Keywords: Laparoscopy, Mast cell infiltration and degranulation, Extracellular matrix thickness, Intra-abdominal adhesion

BACKGROUND

Carbon dioxide (CO_2) insufflation in laparoscopic causes: A. mesothelial morphological changes[1], B. structure damage[2], and C. the risk of intra-abdominal adhesion[3]. Tissue damage triggers the inflammatory response, mast cell infiltration, and degranulation that are thought to stimulate adhesion. The study about the effect of mast cells on the incidence of intra-abdominal adhesion is still rarely done.

Mast cells are specific[4], mature in the tissues, and forms 10% of the mesothelium immune cell population[5]. Laparoscopic causes mast cell infiltration and degranulation. Release of histamine, tryptase, and chymase due to mast cell degranulation[6] are thought to play a role in intra-abdominal adhesion.

Our study aimed to prove the correlation of mast cell infiltration and degranulation to intra-abdominal adhesion after laparoscopic.

METHODS

Animals

According to the principles of experimental animals, i.e., 3R5F (Replacement; Reduction; Refinement; Freedom of hunger and thirst; Freedom from discomfort; Freedom of pain, injury or disease; Freedom to fear and distress; Freedom to express natural behaviour)[7,8], 30 males[9], 200-250 g and 20-25 weeks Sprague-Dawley rats were randomized divided into a control group and four intervention groups. The rats were treated in standard breeding-housing (maintained 20 ± 2^0 C temperature, 12 h light/dark cycle), health monitor, and 7 days of acclimation[10]. The control group (n = 6) did not receive pneumoperitoneum. The intervention groups P 5 mmHg, P 8 mmHg, P 10 mmHg, and P 12 mmHg (all n = 6) were given 5, 8, 10, and 12 mmHg CO_2 pneumoperitoneum, respectively[11].

Laparoscopic procedures

Laparoscopic was done in sterile conditions by shaving and betadine disinfection. Pneumoperitoneum used standard CO_2 and CO_2 automatic insufflators (Gimmi, Gimmi®GmbH, Germany, 2000).

Sample collection

Decapitation was used to sacrifice the rats on the 7th-day after laparoscopic[12]. The greater-omentum, mesenterium, and peritoneum were collected, stained, and evaluated with toluidine-blue for infiltration and degranulation analysis in 100xmagnification[13]. The peritoneum was stained with Masson trichrome to evaluated extracellular matrix (ECM) thickness in 40xmagnification[14]. Three pathologists who were blinded performed evaluations independently used CX31 microscope (Olympus Co., Ltd. Tokyo, Japan) in the U-TV1X-2 lens. Used GraBee version 2.0.0, histological images were captured.

Mast cell infiltration and degranulation histological analysis

The greater-omentum, mesenterium, and peritoneum were collected, washed in saline, layered in object-glass, dried at 70°C for 3 min, and stained with toluidine blue for 5 min[13]. The percentage of mast degranulation is the ratio between the degranulated mast cells and the total number of mast cells.

Mast cell histamine and protease analysis

The peritoneal fluid Mast cell histamine and protease levels were measured using a commercial kit the enzyme-linked-immunosorbent-assay (ELISA). Histamine and protease level used Cloud-clone corp. ELISA Kit for Histamine (HA) for pan-species CEA927Ge[15],

Tryptase (TPS) for Rat SEB070Ra[16], and Chymase-1 Mast Cell (CMA1) for Rat SEG515Ra[17], respectively.

Extracellular Matrix Thickness and intra-abdominal scoring

The ECM thickness was measured using the Masson trichrome stain, based on Skytec TRM-1-IFU's collagen Trichrome Stain (Connective Tissue Stain)[18] collagen deposition and quantified with ImageJ software[19]. Modified intra-abdominal adhesion scoring[2] for laparoscopic was used.

Statistical analysis

Our study presented as numbers, percentages, mean ± standard deviation, and median (minimum-maximum), Data performed normality (using Kolmogorov–Smirnov, and Shapiro–Wilk), homogeneity test (using Levine's), and data transformation methods (power > 1, inverse, log10, and square root). One-way ANOVA and post-hoc LSD tests used for normally-homogeneously distributed data. Welch Robust Test of Equality of Means and the post-hoc Games-Howell test used for normally distributed but non-homogeneously data. Kruskal-Wallis and post-hoc Mann-Whitney test used for non-normally distributed data. Bivariate Linear Regression was used to analyze the effect of mast cell degranulation with intra-abdominal adhesion. A confidence interval of 95% (α=0.05) and the analysis used SPSS version 23.0 and Microsoft Excel 2010.

RESULT

Mast cell infiltration and degranulation

Mast cell infiltration was statistically significantly higher in the intervention groups at a 10 mm Hg than in the control group (61.67±16.66 vs. 62.5±21.58 vs. 69.83±18.35 vs.

104.33±27.75 vs. 107.5±141.28; 53.5 [range, 24-67] vs. 56.5 [range, 39-69] vs. 53 [range, 38-57] vs. 73 [range, 60-85] vs. 68 [range, 46-105]; 44.5±5.68 vs. 59.67±4.03 vs. 65.67±10.01 vs. 89.83±14.74 vs. 90.33±3.88, in the greater-omentum, peritoneum and mesenterium, respectively for control, 5 mm Hg, 8 mm Hg, 10 mm Hg and 12 mm Hg groups, respectively, $p<0.05$.

Mast cell degranulation was statistically significantly higher in the intervention groups at a 10 mm Hg than in the control group (11.8±9.47 vs. 38.76±32.1 vs. 40.97±19.95 vs. 52.03±29.56 vs. 54.15±26.58; 9.95±5.28 vs. 8.12±0.76 vs. 7.24±1.54 vs. 75.69±2.10 vs. 82.13±10.22; 3.24 [range, 0-17] vs. 83.26 [range, 72.31-100] vs. 83.23 [range, 76.97-100] vs. 94.55 [range, 89.86-97.76] vs. 93.44 [range, 71.43-100], in the greater-omentum, peritoneum and mesenterium, respectively for control, 5 mm Hg, 8 mm Hg, 10 mm Hg and 12 mm Hg groups, respectively, $p<0.05$.

Mast cell histamine and protease levels

There was a statistically significantly higher levels in peritoneal fluid histamine and tryptase between the 10 mm Hg intervention than in the control group, 0.04±0.02 vs. 0.03±0.02 vs. 0.04±0.035 vs. 0.50±0.35 vs. 0.41±0.41; 0.48±0.02 vs. 0.56±0.07 vs. 0.53±0.17 vs. 0.69±0.11 vs. 0.65±0.05, respectively, $p <0.05$. There was no statistical difference in an increase in chymase levels in the intervention groups than in the control group.

Extracellular Matrix Thickness and intra-abdominal scoring

There was a statistically significant thicker in the ECM between the intervention groups over 10 mm Hg than in the control group, 10.25 [range, 8.7-12.1] vs 37.15 [range, 31.3-

43.7] vs 40.05 [range, 33.2-44.4] vs 71.3 [range, 66.7-85.2] vs 48.4 [range, 34.5-50.3], respectively, $p < 0.05$.

There was a statistically significant higher in intra-abdominal scoring between the intervention groups over 10 mm Hg than in the control group, 0 vs. 3.5 [range, 0-4] vs. 4 [range, 0-5] vs. 4 [range, 0-4] vs. 4.5 [range, 4-5], respectively, $p < 0.05$.

Relationship of mast cell degranulation and intra-abdominal adhesion

There was a significant correlation between the mast cell infiltration and degranulation of mesothelium, peritoneum, greater-omentum with intra-abdominal adhesion; except mast cell infiltration of the greater-omentum ($p<0.05$).

DISCUSSION

Laparoscopic pneumo-peritoneum causes ischemia-reperfusion injury, especially during desufflation, oxidative stress, and cell damage[20,21]. Cell damage triggers the production of Damage Associated Molecular Patterns (DAMPs) and inflammatory responses[22,23]. Mast cells and other innate immune cells will be active as homeostasis[20]. Mast cells have a unique feature compared to other inflammatory cells[24], mature in tissue, have a longer life, and play a role in the fibrosis process[25]. The pathological conditions were caused the excessive mast cell infiltration and degranulation[26–28]. Our study proved an increase in mast cell infiltration, degranulation, histamine, and tryptase levels in laparoscopic pressure of 10 mm Hg.

The laparoscopic pneumo-peritoneum is non-immunological and physical stimulation[29] and causes mast cell degranulation[17]. Hypoxia triggers anaerobic respiration, ATP deficiency, and results in interference of the mast cell membrane canal that is depending ATPase. This

mechanism disrupts water, ion, and cell homeostasis[30]. Hypoxia causes the activation of the C3a and C5a molecules and activates the G Protein-Coupled Receptors (GPCR) receptors resulting in degranulation[31]. The pressure and cold of CO_2 pneumo-peritoneum cause interference to the Ca^{2+} channel of mast cell[29]. Lipid, protein, and deoxyribonucleic acid (DNA) peroxidation due to Reactive Oxygen Species (ROS) causes mast cell degranulation[32]. Mast cells are not-excitable immunological cells that sensitive to physical trauma[33-34]. TRPC Ca2+ channel is sensitive to temperature changes. CRAC[35] and TRPV4[36] are mechanosensitive (MS) channels that sensitive to pressure. VDAC mitochondria Ca^{2+} channel also regulated cytoplasmic levels and caused mast cell degranulation[29].

Degranulation of mast cells releases histamine and proteases. Mast cells histamine, and proteases are high in fibrosis areas[37]. Histamine causes vascular vasodilation and increases molecular cell adhesion, modulates the migration and proliferation of fibroblasts[25]. Mast cell tryptase and chymase increases TGF-β activity decreases the cell tight junction affinity and has the potential to be a pro-fibrotic protein[25,38]. TGF-β triggers mesothelial-transformation, increasing the ECM thickness[39] and leads fibrosis[40]. Tryptase and chymase are the angiogenic factor[25] and trigger ECM thickness. Chymase results in the degradation of the enzymes' vitronectin and fibronectin, transforming pro-MMP9 into active forms and modulating the thickening of MES[6]. Tryptase causes degradation of type 4 collagen as the main structure of the basement membrane[41]. Tryptase and chymase inhibit the fibrinolysis enzymes (tPA and uPA), increasing fibrin[25]. They activate the PAR-2 receptor causing degradation of the cell junction component, which causes mesothelial release from the basement membrane[42]. Different from the Berdun et al., research[17], our study found no

significant increase in chymase levels. It was suspected that the mast cell chymase population is lower than tryptase. The suspect was associated with the specifics trauma of laparoscopic.

Our study proves an increase in the extracellular matrix and intra-abdominal scoring in laparoscopic over 10 mm Hg. The ECM is a 3-dimensional structure consisting of collagen, enzymes, glycoproteins (proteoglycans), and extracellular vesicles (DNA, RNA, and Matrix-bound Nano vesicles / MBVs)[43,44]. The effect of laparoscopy on ECM is multi-factorial on the 3-dimensional structure of ECM, including mast cell degranulation[45]. Laparoscopic triggers proliferation, differentiation, migration, and formation of ECM towards fibrosis, due to an imbalance of the coagulation and fibrinolysis process[46].Tryptase inhibits the fibrinolysis enzymes (tPA and uPA) and increases fibrin. Histamine causes vasodilation and increases molecular cell adhesion and modulates the migration and proliferation of fibroblasts[25].

There was a correlation in mast cell infiltration and degranulation with intra-abdominal scoring; except mast cell infiltration of the greater-omentum. Intra-abdominal adhesions such as wall-off are the first response from the nearest structures[47]. This mechanism explains that the initial response begins in the mesentery and peritoneum, and the greater-omentum as the next response.

CONCLUSIONS

Our study proved that laparoscopy results in mast cell degranulation increased intra-abdominal adhesion. Mast cells degranulation releases histamine and proteases, and trigger

intra-abdominal adhesion. The next research on mast cell stabilizers promises in preventing intra-abdominal adhesion

Ethical approval

The Animal Experimentation Ethical Committee, Research Center, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia has approved our research (No.282/KEPK-FK.UNLAM/EC/VII/2019). The experiments were conducted in the Chemical/Biochemical Laboratory, the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia

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Author contribution

HP, G, ZN, IKO, KM, BP, MAW and EW conceived the study. HP drafted for study design, data analysis, writing the manuscript. IKO led the anatomic pathology analysis. G, ZN, IKO, KM, BP, MAW, and EW critically revised for study design, data analysis, and the manuscript for valuable intellectual content. All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work appropriately investigated and resolved.

Conflict of interest: No conflict of interest

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396