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MCP-1 INDUCED HISTAMINE RELEASE FROM MAST CELLS IS ASSOCIATED WITH THE FORMATION OF INTERSTITIAL CYSTITIS IN RATS

Hypothesis / aims of study
Interstitial cystitis (IC) is a complex disease which is characterized with induction of mast cells (MC) degranulation. Currently we know some factors (including inflammatory factors, neuropeptides, trauma, and toxins) ion, will lead to stimulate the activation of MC, releasing the histamine (HA), protease, vascular active substances and inflammatory factors, thereby increasing the organization of the inflammatory and fibrosis. The role of MC in IC progression is to be affirmed. Recently, The monocyte chemoattractant protein-1(MCP-1) may be involved in the promotion of MC activation and degranulation, releasing the HA and a series of inflammatory factors. However, it remains unclear whether the increase MCP-1 is involved in MC releasing HA in IC process, the exact roles of MCP-1 in IC development are still unknown. This study is to observe the MCP-1 and HA expression levels in IC rats bladder tissue and urine induced by intravesical instillation of protamine sulfate (PS) and lipopolysaccharide (LPS), and the mechanism of MCP-1 in the IC and vitro experiment.

Study design, materials and methods
Animal experiments: Female SD rats, weighing 250 ~ 300g, were divided into the IC and control groups. IC group were treated with 10 mg / ml PS and 750 ug / ml LPS by intravesical instillation, and in PBS was used for bladder instillation in the control group. All rats were killed three days later, the bladder tissue and urine of rats were stored. By ELISA and Western Blot, the expression levels of MCP-1 and HA were detected in the rat bladder tissue and urine. Bladder tissue inflammatory score was used by HE staining, MC degranulation count was used by special staining. MCP-1, CCR2 receptor and MC were analysised by immunohistochemical method and immunofluorescence method. **Vitro experiments:** Human bladder epithelial cells (BEC) cultured in vitro, is divided into negative control group and different concentrations of LPS group to stimulate the BEC (10, 100, and 500 ng / ml), at different times after treatment (24h, 48h and 72h) expression levels of MCP-1 were detected by ELISA method. Flow cytometry was used to detect expression of CCR2 receptor in mast cells (MC) surface cultured in vitro, and which divided into negative control group and different concentrations of MCP-1 group to stimulate the MC (10 and 100 ng / ml), at different times after treatment (24h, 48h and 72h), expression levels of HA were detected by ELISA method.

Results
Animal experiments: By the method of ELISA and Western-Blot, the expression levels of MCP-1 and HA in the bladder tissue and urine of IC rats were significantly increased to control group ($P < 0.01$). Though HE staining, the IC group and control group mononuclear inflammatory cell count were respectively (76.5 ± 9.8) and (18.5 ± 9.8) / field ($P < 0.01$). More inflammatory cell infiltration in the bladder mucosa, edema mucosa, congestion and hemorrhage were seen by HE staining. With MC special staining, degranulation MC count in the IC group and control group count were (6.4 ± 3.1) and (0.7 ± 0.3) / field ($P < 0.01$). Immunohistochemical methods found MCP-1 and CCR2 receptor have a higher expression in the bladder epithelium, and more MCP-1 were found gathering round MC surface by immunofluorescence. **Vitro experiments:** Through the detection of flow cytometry, more CCR2 receptor expression was found on MC surface. Different concentrations of LPS stimulation in vitro BEC were detected by ELISA. The secretion of MCP-1 were significantly higher ($P < 0.01$), and concentration-dependent. When the LPS concentration of 100 ng / ml, secretion of MCP-1 could reach the maximum effect, which can maintain to 48h. The different concentrations of MCP-1 to stimulate the in vitro culture of MC were detected by ELISA. HA secretion were significantly higher ($P < 0.01$), and can maintain to 48h. The secretion of HA was no significant differences between 10 and 100 ng / ml concentrations of MCP-1 ($P > 0.05$).

Interpretation of results
The mechanism of MCP-1 in the IC process may be, some toxic substances in the urine (such as: LPS) stimulate the bladder epithelial cells to produce more MCP-1. MCP-1 could combined with the MC surface through the CCR2 receptor, induced activation of MC, which could release much HA and other inflammatory factors, thereby aggravating the pathological process of inflammatory and fibrosis in IC.

Concluding message
The up-regulation of MCP-1 in IC is a causative factor for induction of histamine release from mast cells through CCR2 in bladder tissue, which results in the development of IC.

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Disclosures

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