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ORIGINAL ARTICLE

Patterns of matrix metalloproteinases and transforming growth factor-beta 1 expression during peritoneal repair in chlorhexidine induced peritoneal fibrosis mice

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KEYWORDS
Chlorhexidine gluconate;
Matrix metalloproteinase;
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Tissue repair;
Transforming growth factor-beta 1

Summary *Background/Purpose:* Recovery from peritoneal fibrosis (PF) involves the digestion of accumulated collagens and remodeling. Matrix metalloproteinases (MMPs) may play an important role in repair. The role of MMP-13, an important component in the MMP cascade, in PF is still unclear. We examined the sequential expression of MMP synthesis during repair in a PF mouse.
Methods: Forty-eight mice at 8 weeks of age were given an intraperitoneal injection of 0.1% chlorhexidine gluconate (CG) for 3 weeks. Control mice were injected with the same dosages of 15% ethanol dissolved in saline. These mice were sacrificed, and anterior abdominal walls were obtained on days 21, 28, 35, 42, 49, and 56. Gene expressions of MMP-2 and -13, tissue inhibition of metalloproteinase-1 (TIMP-1) and -2, MT1-MMP, transforming growth factor-beta 1, and collagen types I and III were analyzed by real-time polymerase chain reaction. MMP-13 enzyme activity was also measured. In immunohistological evaluation, MMP-13 expressing cells were examined.
Results: Thickening of the peritoneum and marked infiltration of monocytes were induced by CG, and the alterations remained until 7 days after cessation of CG. Then tissue repair rapidly advanced. Synthesis of collagen types I and III, MMP-2, TIMP-1 and -2, and transforming growth factor-beta 1 in the injured peritoneum was significantly increased until day 28. These increments were preceded by an increase of MMP-13 synthesis and activity after cessation of CG. Some infiltrating macrophages in the thickened peritoneum showed MMP-13 expression early after cessation of CG.

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Conclusion: MMP-13 was synthesized by infiltrating monocytes early in the repair process in the CG-induced PF mouse. After cessation of stimulant, increase of MMP-13 synthesis may act as an inducer of an efficient degradation cascade in collagen-rich peritoneal tissue.

背景: 腹膜纖維化的復原涉及膠原蛋白堆積的消化與其後的重塑，其中，基質金屬蛋白酶(MMPs)在組織修復期間可能佔有重要的角色。作為MMP級聯中的重要一環，MMP-13在腹膜纖維化上的角色仍然未明。本研究透過腹膜纖維化(PF)的小鼠，調查了在腹膜修復期間MMP生成的表現順序。
方法: 研究材料為48隻年齡8週的小鼠，先接受每天共3週的0.1%葡萄糖酸氯己定(CG)腹膜內注射；另外對照組的注射則採用相同容量or劑量含15%乙醇的鹽水。其後陸續宰殺小鼠以取得第21、28、35、42、49及56天的前腹壁檢體，並採用實時PCR測量以下的基因表現：MMP-2-13、TIMP-1-2、MT1-MMP、TGF-β(1)、及I III型膠原蛋白。此外亦同時測量MMP-13之酵素活動，並對表現MMP-13的細胞作免疫組織學評估。
結果: 在CG誘導下，可見腹膜增厚及單核球的明顯浸潤，直至停止CG後7天，其後可見組織修復的快速進行。在停止CG後的受損腹膜中，首先可見MMP-13生成與活動的增加，其後亦可見I III型膠原蛋白、MMP-2、TIMP-1-2、及TGF-β(1)生成的明顯增加，直至第28天。在停止CG後的早期，可見增厚腹膜中有若干巨噬細胞具MMP-13表現。
結論: 在CG所致的PF小鼠中，腹膜修復早期已可見浸潤的單核球產生MMP-13。在停止刺激後，在富含膠原蛋白的腹膜環境中，MMP-13似乎可促使一個高效降解級聯的進行。

Introduction

Marked thickening of the peritoneum and vasculopathy in the submesothelial compact zone (SMC) were reported in long-term peritoneal dialysis (PD) patients.[1] The key factors of peritoneal regeneration after cessation of PD are still unclear. Recovery from peritoneal fibrosis (PF) to a normal peritoneum involved the destruction of accumulated collagens and excess matrix and tissue remodeling in the peritoneum. Matrix metalloproteinases (MMPs) may play important roles in such events.

Elevated expression of collagen type III was observed in early or active lesions of pulmonary fibrosis, and expression of collagen type I increased in chronic pulmonary fibrosis.[2] In scar formation in the healing process of full-thickness excisional wounds, the synthesis of collagen types I and III increased during the later phase of wound healing and peaked at around 2 weeks after surgery.[3] Suga et al[4] and Fukuda et al[5] reported that idiopathic pulmonary fibrosis cases showed a predominant expression of MMP-9, whereas nonspecific interstitial pneumonia and bronchiolitis obliterans organizing pneumonia cases showed a predominant MMP-2 expression in bronchoalveolar lavage fluid and in tissues. MMP-1 and -9 expressions were elevated during granulation tissue formation and re-epithelialization, whereas in the remodeling phase, MMP-2 and MMP-9 messenger RNAs (mRNAs) were increased in experimental full-thickness wound healing.[3] Collagenases MMP-1, -8 and-13 can cleave collagens into specific fragments for an efficient degradation by gelatinase,[6] and MMP-13 especially can activate latent transforming growth factor-beta 1 (TGF-β1) on cell surfaces.[7] Once TGF-β1 has been activated, it increases collagen synthesis,[8,9] tissue inhibition of metalloproteinase-1 (TIMP-1),[10] and MMP-2 activity through the inhibition of TIMP-2.[11] Furthermore, TGF-β1 can suppress expression of collagenase.[12] Since these proteolytic activities are regulated by several mechanisms including regulation of gene expression by cytokines or hormones, and extracellular cleavage of the proenzyme to the active form by membrane-type MMP (MT-MMP) inhibition by TIMPs, the qualitative pattern and quantitative levels of MMPs vary among tissues, diseases, tumors, inflammatory conditions, and cell lines.[13]

The objective of the present study was to find a clue on how to break out of the vicious cycle in long-term PD patients with severe PF. In this study, we observed serial and temporal alterations of collagens, MMPs and TIMPs and TGF-β1, associated with tissue fibrosis and remodeling in the process of peritoneal repair after repeated intraperitoneal injection of chlorhexidine gluconate (CG) in mice. We examined the relationship between these alterations and morphological findings in peritoneal repair.

Materials and methods

Animal model

Fifty-one C57BL/6 male mice were purchased at 8 weeks of age from CLEA Japan Inc. (Tokyo, Japan). They were housed in a specific pathogen free (SPF), light- and temperature-controlled room. They had free access to laboratory chow and tap water in standard rodent cages. All animal studies were performed according to the guidelines of the Ethics Review Committee for Animal Experimentation of Juntendo University Faculty of Medicine. Forty-eight mice were given an intraperitoneal injection (i.p.) of 0.35 mL of 0.1% CG and 15% ethanol dissolved in saline three times a week for 3 weeks.[14] The mice were injected with the same dosages of 15% ethanol dissolved in saline without CG as control ($n = 3$). These mice were sacrificed, and the anterior abdominal walls were collected on days 21, 28, 35, 42, 49, and 56 ($n = 8$ in each day).

Histological assessment

Serial morphological changes in the peritoneum after repeated CG injection were evaluated. The parietal abdominal walls were fixed in 10% neutral-buffered formaldehyde and embedded in paraffin. Then 4-μm sections