Pentraxin3 expression in a rat model of peritoneal dialysis

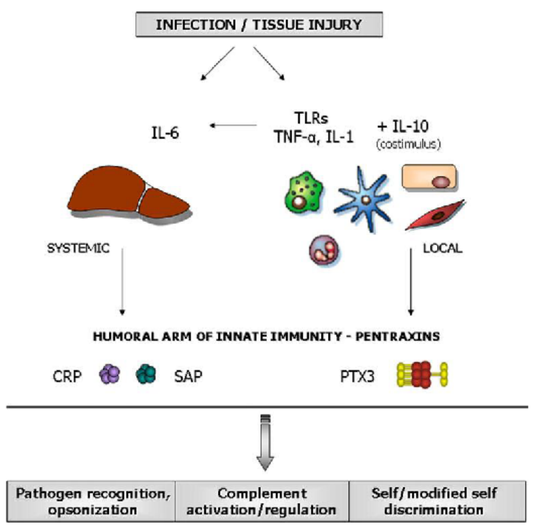
Nana Ishimatsu[1], Tetsu Miyamoto[1], Hiroyuki Morimoto[3], Junichi Nakamata[1], Ryoko Baba[3], Hiromichi Ueno[1], Yoko Nakano[1], Kenichiro Bando[2], Yumi Furuno[1], Yutaka Otsuji[1], Masahito Tamura[2]

[1] The Second Department of Internal Medicine, [2] Kidney Center, and
[3] Department of Anatomy, University of Occupational and Environmental Health

Background

Long-term peritoneal dialysis (PD) is known to result in functional and structural alterations in the peritoneal membrane. Continuous exposure to peritoneal dialysis fluid (PDF) drives pathological responses. Persistent intraperitoneal local micro-inflammation exacerbates the histological deterioration, worsening the treatment outcome of PD [(1)~(3)].

Pentraxin3 (PTX3)



Pentraxin3 is a multifunctional soluble pattern recognition receptor that modulates immune inflammatory responses. In contrast to C-reactive protein (CRP), a systemic inflammation marker that is primarily generated by hepatocytes, PTX3 is produced at sites of inflammation by a wide range of various cell types. PTX3 is produced in response to IL-1 and TNF-α by vascular smooth muscle cells, endothelial cells, macrophages, fibroblasts, etc. The PTX3 levels reflect the amount of local inflammation in blood vessels more accurately than levels of CRP [(4)].

Materials & Methods

Materials

(1) PD model rat

The 8-weeks-old Wistar rats were instilled with 20 ml of conventional lactate-buffered PDF containing 3.86% glucose or saline twice a day for 8 weeks.

① Control: no injection (n=6) ② Saline (n=5) ③ PDF (n=6)

(2) Cultured Cells

We used three types of cells.

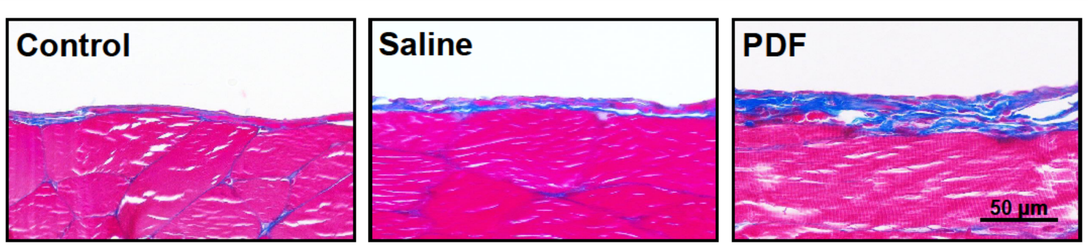
① Rat peritoneal mesothelial cells (RPMCs)
② Mouse macrophage-like cells (RAW 264.7)
③ Mouse fibroblasts (NIH-3T3)

Glucose was added into the culture medium at various concentrations for 4 hours.

Methods

Masson Trichrome stain, RT-PCR, Real-time PCR, *in situ* hybridization, Western Blotting, Immunohistochemical Stain

Histological Analysis of Peritoneum



A histological analysis showed increases in submesothelial thickness in the PDF-treated rats. Such thickening was associated with connective tissue accumulation that stained blue with Masson Trichrome stain.

Reference

(1) Williams JD et al. J Am Soc Nephrol (2002) 13: 470-479
(2) Velloso MSS et al. Clin Chim Acta (2014) 430: 109-114
(3) Williams JD et al. Kidney Int (2003) 88: S43-49
(4) Livija D et al. Cell Tissue Res (2011) 343: 237-249

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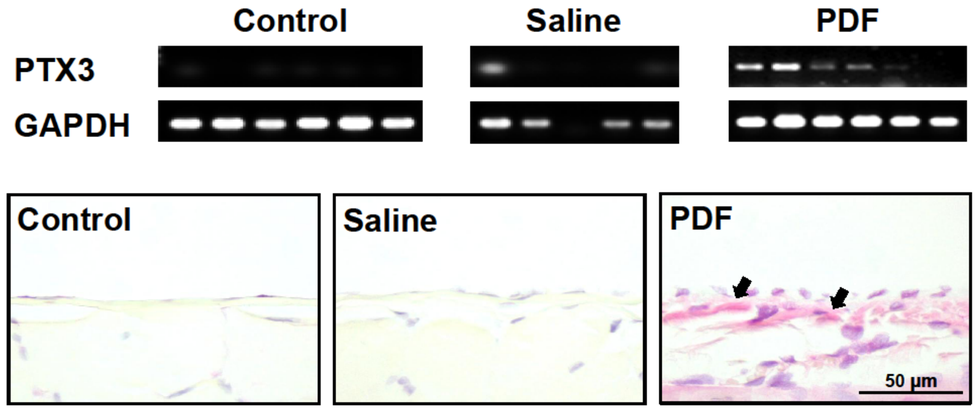
All the authors declare no conflict of interest.

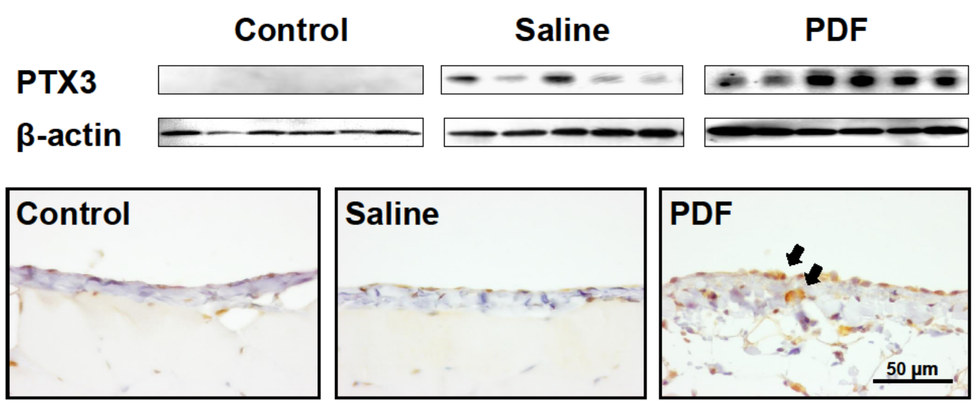
PTX3 mRNA Expression by RT-PCR and *in situ* Hybridization



RT-PCR; PTX3 mRNA was detected in 5 out of 6 rats in the PDF group, 2 out of 5 in the saline group, and none in the control group. Real-time PCR analysis showed that PTX3 expression was significantly higher in PDF-treated rats (107.92 ± 50.83 fold) and saline-treated rats (0.85 ± 028 fold) compared to the control ($P < 0.001$) (Data not shown).

***In situ* Hybridization;** PTX3 mRNA was detected by anti-sense probes only in the peritoneum of the PDF-treated rats (arrow). No PTX3 expression was seen in the peritoneum of the rats in the control and saline-treated groups.

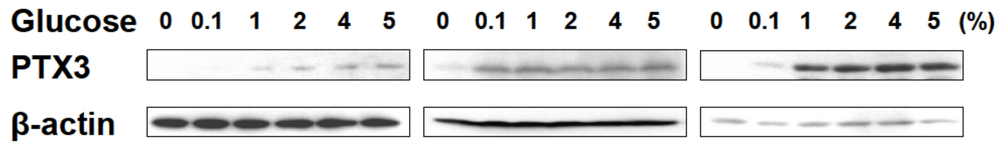
PTX3 Protein Expression by Western Blotting and Immunohistochemical Staining



Western Blotting; PTX3 protein was expressed in all samples of the PDF group. Although PTX3 was detected in the saline group, the expression levels were lower compared with the PDF group. No expression was observed in the control group.

Immunohistochemical Stain; PTX3 protein was detected in the mesothelial cells and submesothelial connective tissue of the PDF-treated rats (arrow). No PTX3 expression was seen in the rats of the control and saline-treated groups.

PTX3 Protein Expression in Cultured Cells by Western Blotting



PTX3 protein expression was detected in RPMCs, RAW 264.7 and NIH-3T3 cells treated with glucose.

Conclusions

(1) PDF-treated rats showed
- the submesothelial thickning
- the expression of PTX3 mRNA and protein
- PTX3 expression in the peritoneal tissue

(2) Rat peritoneal mesothelial cells, macrophages and fibroblasts showed the expression of PTX3 protein by loading the glucose

PTX3 expression was induced by the continuous exposure of the conventional PDF. High glucose may be involved in the mechanism of PDF-induced local micro-inflammation in the peritoneum.

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L2) Dialysis. Peritoneal dialysis.
Tetsu Miyamoto
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