Does endothelin B receptor deficiency ameliorate the induction of peritoneal fibrosis in experimental peritoneal dialysis?

Philipp Kalk, Matthias Rückert, Michael Godes, Karoline von Websky, Katharina Relle, Hans-Hellmut Neumayer, Berthold Hocher and Stanislao Morgera

1Center for Cardiovascular Research/Department of Pharmacology and Toxicology, Charité, Berlin, Germany, 2Department of Nephrology, Charité, Berlin, Germany and 3Institute of Vegetative Physiology, Charité, Berlin, Germany

Correspondence and offprint requests to: Stanislao Morgera; E-mail: Stanislao.morgera@charite.de

*Both authors contributed equally to the publication.

Abstract

Background. Peritoneal fibrosis is a serious complication of peritoneal dialysis (PD); however, the mechanisms are poorly understood. The endothelin system exhibits potent pro-fibrotic properties and is known to be stimulated in peritoneal fibrosis. Thus, our study aimed at elucidating the impact of the endothelin B (ETB) receptor on peritoneal membrane thickening by means of an ETB-deficient rat model (ETB−/−) in experimental PD.

Methods. Wild-type (WT) and ETB−/− rats were randomly allocated to four groups (each group n = 10): (i) WT Sham, (ii) WT PD, (iii) ETB−/− Sham and (iv) ETB−/− PD. All animals underwent surgical implantation of a port for intraperitoneal administration and 1 week of habituation to the procedure by administration of 2 ml of saline once daily. Afterwards, all animals were switched to 12 weeks of 15 ml of saline (Sham groups) or commercially available PD fluid containing 3.86% glucose (PD groups) administered twice daily. Afterwards, animals were sacrificed, and samples from visceral as well as parietal peritoneum were obtained. The samples were stained with Sirius-Red, and at 10 different sites per sample, peritoneal membrane thickness was measured using computer-aided histomorphometry devices.

Results. Mean peritoneal membrane thickness was increased by PD in both WT and ETB−/− rats versus respective Sham controls (WT Sham: 22.3 ± 0.7 µm/ETB Sham: 22.3 ± 0.9 µm versus WT PD: 26.5 ± 1.5 µm/ETB PD: 28.7 ± 1.2 µm; P < 0.05, respectively). However, no difference in peritoneal membrane thickness was detected between WT PD and ETB−/− PD groups.

Conclusion. Our study demonstrates that PD increases peritoneal membrane thickness in a rat model, but deficiency of the ETB receptor has no detectable impact on this process.

Keywords: endothelin; ETB receptor; peritoneal membrane thickening

Introduction

Peritoneal fibrosis is a common complication in patients with end-stage renal disease on peritoneal dialysis (PD) [1]. The pathophysiological mechanisms involved in the process are only partially known. However, endothelin-1 (ET-1) is a potent pro-inflammatory and pro-fibrotic mediator, as its major biological effects include the induction of mitogenesis of fibroblasts, smooth muscle cells and myocytes, activation of neutrophils and the induction of fibronectin as well as chemotaxis of fibroblasts [2]. It acts via two receptors: the endothelin A (ETA) and endothelin B (ETB) receptor [3,4].

© The Author 2009. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.

For Permissions, please e-mail: journals.permissions@oxfordjournals.org
Both receptors are involved in the mediation of inflammation [5] and fibrosis [6]. In fibrotic diseases, often a shift is observed from a normal ETA/ETB tissue distribution to a diseased distribution favouring the ETB receptor [7–9]. Apart from being the cause of tissue fibrosis, this shift can also be interpreted as a counter-regulatory mechanism as the ETB receptor acts as a clearance receptor for ET-1 [10]. Furthermore, the ETB receptor mediates the production of nitric oxide [11] which in turn downregulates ET-1 production.

Concerning the role of ET-1 in peritoneal fibrosis, we demonstrated earlier in a human study that, indeed, the peritoneal ET-1 release is stimulated with increasing dwell volume in PD [12]. In order to assess the impact of ET-1 in mediating peritoneal fibrosis, we performed a cell culture study with human mesothelial cells [13]. We demonstrated that under conditions of increasing osmolarity or fluid stress indeed ET-1 release and collagen I expression was increased in human mesothelial cells, whereas presence of a dual ET receptor antagonist inhibited those effects. However, as a dual ET receptor antagonist was used in this study, the contribution of each specific ET receptor to peritoneal scarring remained unclear. A recent study by Shimizu et al. [14] also using cultured human mesothelial cells indicated a role for the ETB receptor in fibrous matrix protein production. Thus, we designed the present study to assess the impact of the ETB receptor on peritoneal fibrosis in a rat model of PD.

Materials and methods

Study design

Animal studies were carried out in accordance with German law governing the use and care of laboratory animals. Animals were housed under standardized conditions and with water and food ad libitum. ETB receptor-deficient rats (ETB−/−) and wild-type controls (WT) were kindly provided by M. Yanagisawa. As the complete knockout of the ETB receptor is lethal due to intestinal aganglionosis (Hirschsprung's disease), the model we used is a transgenically rescued spotting lethal rat which carries a naturally occurring deletion of the ETB receptor. In our model, this rat is rescued by a transgenic ETB receptor gene under control of a dopamine-beta-hydroxylase promoter allowing expression of the ETB receptor mostly in intestinal tissue in order to prevent aganglionosis [15]. As it has not yet been demonstrated that, in peritoneal tissue of this model the ETB receptor is absent, we performed real-time PCR (rt-PCR) in order to confirm the absence as described in previous studies [16].

During the study, Wistar rats were randomly allocated to four groups (each group: n = 10):

1. WT Sham
2. WT PD
3. ETB−/− Sham
4. ETB−/− PD

All animals underwent surgical implantation of a port (ROP, Access Technologies, Illinois, US; Figure 1) for intraperitoneal application of PD fluid (PD groups) or saline (Sham groups) as follows: After animals were put in general anaesthesia, two incisions were made: First, incision was performed dorsally at the neck, and a pocket for the port was built. Second incision was made at the left side of the abdomen. Afterwards, from the dorsal neck incision to the abdominal incision, a subcutaneous tunnel was prepared. The port

![Fig. 1. Implanted port system and i.p. administration of either PD fluid or saline (i.p. administration was done by transcutaneous puncturing of the port).](https://academic.oup.com/ndt/article-abstract/25/5/1474/1838891)

![Fig. 2. Photograph of rt-PCR results in lung, cerebral cortex and peritoneal tissue (this demonstrates the absence of the ETB receptor in peritoneal tissue of our rat model).](https://academic.oup.com/ndt/article-abstract/25/5/1474/1838891)
was put into the pocket at the neck, and the catheter was inserted through the tunnel to the abdominal incision where the peritoneum was opened, and the tip of the catheter was inserted into the peritoneal cavity.

Afterwards, all incisions were closed with sutures. Afterwards, 1 week for habituation to the port management was given by administration of 2 ml saline once daily (Figure 1). After habituation, animals were switched to 12 weeks of 15 ml of saline (Sham groups) or commercially available PD fluid containing 3.86% glucose (PD groups) administered twice daily. Throughout the study, antibiotic prophylaxis was carried out using staphylococcal (2.5 mg/day) and gentamycin (0.04 mg/day). At study end, PD fluid was stored at −80°C. ET-1, fibronectin, vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) were analysed later by the appropriate ELISAs provided by Immundiagnostics, Bensheim, Germany. Thereafter, blood was taken, the animals were sacrificed and tissue samples from visceral as well as parietal peritoneum were obtained. Tissue samples were stained with Masson’s trichrome and at 10 randomly chosen sites per sample peritoneal membrane thickness was measured using a light microscope combined with a PowerMac and image processing software (ImageJ, shareware from the National Institute of health).

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Peritoneal Membrane Thickness [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT + Sham</td>
<td>10</td>
</tr>
<tr>
<td>WT + PD</td>
<td>12</td>
</tr>
<tr>
<td>ETB −/− + Sham</td>
<td>14</td>
</tr>
<tr>
<td>ETB −/− + PD</td>
<td>16</td>
</tr>
</tbody>
</table>

Legend: All values are given as mean ± SEM.
*: p < 0.05 vs. WT + Sham
†: p < 0.05 vs. ETB −/− + Sham

![Peritoneal membrane thickness diagram](image_url)

*Fig. 3.* (A) All values are given as mean ± SEM (*P < 0.05 versus WT Sham, †P < 0.05 versus ETB −/− Sham). (B) Microscopic samples of peritoneal tissue in Masson’s trichrome staining of the four study groups, magnitude ×200.
Table 1. Concentration of ET-1 and other fibrosis-related mediators in dialysis fluid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>Sham</th>
<th>PD</th>
<th>ETB−−</th>
<th>Sham</th>
<th>ETB−−</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1 (pg/ml)</td>
<td>1.54 ± 0.97</td>
<td>0.82 ± 0.61</td>
<td>0.48 ± 0.48</td>
<td>0.02 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin (pg/ml)</td>
<td>653.9 ± 17.5</td>
<td>603.4 ± 15.2</td>
<td>659.2 ± 13.1</td>
<td>609.1 ± 28.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>17.3 ± 3.7</td>
<td>14.7 ± 3.4</td>
<td>22.5 ± 3.9</td>
<td>9.6 ± 2.1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β (pg/ml)</td>
<td>462.2 ± 189.7</td>
<td>137.0 ± 135.0**</td>
<td>1466.8 ± 353.7*</td>
<td>868.5 ± 311.3*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are given as means ± SEM.

*P < 0.05 versus WT Sham.

**P < 0.05 versus WT PD.

***P < 0.05, ****P < 0.001 versus ETB−− Sham.
matrix protein production. The discrepancy may be due to the fact that the authors focused on fibronectin whereas other matrix proteins (i.e. collagens) might be more important in peritoneal scarring. In our trial, the lowest fibronectin values were detected in WT rats on PD. We further should keep in mind that cell culture studies are highly artificial compared to the in vivo situation. Based on our findings and the well-known pro-fibrotic actions of the ETA receptor [25,26], we suggest the ETA receptor as a potential pathway in endothelin-dependent peritoneal scarring and warrant further studies to clarify this point.

In summary, our study demonstrates that PD increases peritoneal membrane thickness in our rat model, but deficiency of the ETB receptor has no detectable impact on this process. However, the role of the ETA receptor in peritoneal fibrosis is unknown and should be addressed in further studies.

Acknowledgements. We thank Masashi Yanagisawa for kindly providing the ETB receptor-deficient rats and wild-type controls. We thank Dr. Svetlana Sperling and Prof. Hannelore Ehrenreich, Division Klinische Neuroradiologie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, for performing the rt-PCR experiments. This study was partially supported by grants from the Deutsche Forschungsgemeinschaft (DFG) (HO 1665/5-2) to B.H.

Conflict of interest statement. None declared.

References