Prostaglandin inhibition by intraperitoneal indomethacin has no effect on peritoneal permeability during stable CAPD

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Abstract

Background. Prostaglandins can affect the vascular response and are locally produced in the peritoneal cavity. Prostaglandin inhibition in continuous ambulatory peritoneal dialysis (CAPD) patients during peritonitis using indomethacin intraperitoneally was found to decrease the intrinsic permeability to macromolecules.

Methods. In the present study the effects of prostaglandin inhibition were studied during stable, uninfected CAPD. Two standard peritoneal permeability analyses (1.36% glucose) were performed in 10 stable CAPD patients within 1 week with and without addition of 12.5 mg/l indomethacin. Furthermore, possible effects on the parameters of nitric oxide synthesis were determined. In five other patients a high dose of indomethacin was tested. The night before the indomethacin test, 12.5 mg/l indomethacin was added to the nightdwell and the test was performed with 25 mg/l indomethacin.

Results. In the normal dose indomethacin group, the dialysate concentrations of prostaglandin (PG) 6-keto-PGF_1α and thromboxane (Tx) TXB_2 were significantly lower with indomethacin (IND) compared with the control dwell (C): 6-keto-PGF_1α median 93 (C) vs 7.5 (IND) ng/l, P = 0.006 and TXB_2 12.3 (C) vs 9.0 (IND)ng/l, P = 0.04. The dialysate concentration of PGE_2 was not different during the control dwell (68.5 ng/l) compared with the indomethacin experiment (50.3 ng/l, P = 0.5). The mass transfer area coefficients (MTAC) of nitrate and cGMP, and parameters of nitric oxide synthesis, were similar during both experiments. The MTAC of creatinine and urate were not different with indomethacin: creatinine median 9.5 (C) vs 10.2 ml/min (IND), P = 0.2 and urate 7.2 (C) vs 7.3 ml/min (IND), P = 0.3. Only the MTAC of urea was marginally higher with indomethacin: 16.0 (C) vs 16.6 ml/min (IND), P = 0.04. No differences were found in the clearances of the macromolecules (β2-microglobulin, albumin, IgG and α2-microglobulin. With the high indomethacin dose no inhibition of PGE_2 was found: 69 (C) vs 63 ng/l (IND), not significant. Furthermore, no differences were found in the transport rates of small solutes or proteins. This indicates no effect of indomethacin on the peritoneal surface area and the size-selective permeability to macromolecules. In both groups no effect was found on the transcapillary ultrafiltration and the effective lymphatic absorption rate during the 4-h dwell. Consequently, the net ultrafiltration, the difference between these, did not change.

Conclusions. The indomethacin induced inhibition of the synthesis of 6-keto-PGF_1α and TXB_2 did not lead to alterations in functional parameters of the peritoneal surface area, the intrinsic permeability to macromolecules and fluid kinetics. Therefore, these prostaglandins are not likely to be involved in the regulation of peritoneal transport during stable CAPD.

Keywords: CAPD; indomethacin; permeability; prostaglandins; nitric oxide

Introduction

The transport of solutes and fluid across the peritoneal membrane during continuous ambulatory peritoneal dialysis (CAPD) is determined by its surface area and intrinsic permeability. The effective or functional peritoneal surface area is the part of the peritoneum that is actually involved in the transport of solutes. It is dependent on the splanchnic blood volume and so on the number of perfused peritoneal capillaries [1,2]. These are dynamic factors, implying that the effective surface area is variable. For example, instillation of
dialysis solutions influences the peritoneal transport capacity by vasodilation of the vessels of the peritoneal membrane [3,4]. Consequently, the peritoneal transport characteristics are significantly higher during the initial phase of a dwell [5,6]. It is likely that locally-produced vaso-active mediators will be involved in the regulation of the number and diameter of perfused peritoneal capillaries. Prostaglandins may be among these mediators as they can be synthesized by cultured human peritoneal mesothelial cells [7]. In the peritoneal cavity prostaglandins are locally produced [8,9].

Both the vasodilating prostaglandins PGE2 and 6-keto-PGF12 and the vasoconstricting PGF2 and thromboxane (Tx) TxB2 have been detected in the effluent of stable CAPD patients [10]. During peritonitis, a situation with vasodilation and increased peritoneal permeability, the concentrations of especially the vasodilating prostaglandins in the effluent are markedly elevated [11,12]. Inhibition of local prostaglandin synthesis by intraperitoneal (i.p.) administration of indomethacin during the initial phase of peritonitis reduced the infection induced increased size-selective intrinsic permeability, but had no effect on the parameters of the effective peritoneal surface area [13].

The aim of the present study was to investigate the role of locally produced prostaglandins in the regulation of peritoneal membrane characteristics during stable, non-infected CAPD. This was performed by i.p. administration of the same dose of indomethacin, to stable CAPD patients, that had proved to be effective during peritonitis and also of a higher dose during a longer time period. Furthermore, possible effects on the parameters of nitric oxide synthesis were determined.

Patients and methods

Patients

In 10 stable CAPD patients (four female/six male) two standard peritoneal permeability analyses (SPA) were performed within 1 week for testing the normal dose of indomethacin. The patients had a mean age of 53 years (range 29–75 years). They were treated with CAPD for 5–88 months (median 22 months). The causes for renal replacement therapy were hypertensive nephropathy (three patients), chronic glomerulonephritis (four patients) diabetic nephropathy (one patient), obstructive uropathy (one patient) and systemic lupus erythematosus (one patient). In five other patients (all male) with a mean age of 58 years (range 50–64 years) a higher indomethacin dose was tested. These patients were treated with CAPD for 3–105 months. The causes for renal replacement therapy were hypertensive nephropathy (three patients), chronic glomerulonephritis (one patient) and obstructive uropathy (one patient). All patients performed CAPD using commercially available dialysate (Dianeal®, Baxter BV, Utrecht, The Netherlands). None of the patients had peritonitis at the time of the study or in the four preceding weeks. The protocol was approved by the Committee of Medical Ethics of the University Hospital of Amsterdam and informed consent was obtained from all patients after an explanation of the purpose and methods of the study.

Procedure

The SPAs were performed during 4-h dwell periods [14]. For testing the normal dose of indomethacin, both tests were done with 1.36% glucose dialysate (Dianeal®), 1 day with and the other day without addition of 12.5 mg/l indomethacin. To all test bags dextran 70 (Macrodex®, NPBI, Emmers-Compascuum, The Netherlands) 1 g/l was added to calculate peritoneal fluid kinetics [15]. The sequence of both experiments had been randomized. Prior to instillation of the test solution, the peritoneal cavity was rinsed with 1.36% glucose dialysate. On the indomethacin day, 12.5 mg/l indomethacin was also added to the rinsing bag. Samples were taken from the test dialysate before inflow and 10, 20, 30, 60, 120, 180 and 240 min after instillation of the test solution. To avoid a dead-space effect, 100–200 ml was temporarily drained before each sample was collected. After drainage of the test solution, the abdomen was rinsed again with 1.36% glucose dialysate. Samples from this rinsing bag were used for the calculation of the residual volume. Blood samples were drawn at the start and at the end of the test. Dextan 1 (20 ml, Promite®, NPBI, Emmers-Compascuum, The Netherlands) was given intravenously (i.v.) after the first blood sample to prevent possible anaphylaxis to dextran 70 [16].

For testing the high-dose of indomethacin, 12.5 mg/l indomethacin was added to the night dwell, the night before the indomethacin experiment. Also 12.5 mg/l indomethacin was added to the rinsing bag. The indomethacin-SPA was performed with 25 mg/l indomethacin added to the test bag. The further procedure of the SPA was similar as described for the normal indomethacin dose.

Assays

Total dextran 70 was determined in all dialysate samples by means of high-performance liquid chromatography [17]. Both in plasma and dialysate urea, creatinine and urate were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany). Beta-2 microglobulin was determined on an IMx system using a Microparticle Enzyme Immunoassay (Abbott laboratories, North Chicago, IL, USA). Albumin, IgG and s2-macroglobulin were determined by nephelometry (BN100, Behring, Marburg, Germany). Glucose was measured by glucose oxidase-peroxidase method using an auto analyzer (SMA-II, Technicon, Tarrytown, USA).

Blood and dialysate samples for nitrite, nitrate and cGMP were centrifuged at 1530 g for 10 min and immediately stored at –20°C. Nitrite and nitrate were measured by ion-pair high-performance liquid chromatography. cGMP was measured by an Enzyme Immunoassay (Cayman Chemical Company, Ann Arbor, USA).

The prostaglandins PGE2, 6-keto-PGF1, PGF2a, and TxB2 were determined in dialysate by radioimmunoassays according to the method of Thomas et al. [18] with one modification: the samples were not purified before the analysis, because purification resulted in the same values for all prostaglandins [11]. The tritium labeled tracers were supplied by Dupont (Dreieich, Germany). Antiserum against PGF2 was provided by Dr. J. J. Pratt (Academic Hospital Groningen, The Netherlands). Antiserum against 6-keto-PGF1, PGF2a, and TxB2 were purchased from Cayman Chemical (Ann
Arbor, USA). Cross-reactivity with the other prostaglandins was 4.3% for PGE2, 10.7% for 6-keto-PGF1a, 5.3% for PGF2a and 2.0% for TxB2. The lower detection limits were 18.0 ng/l for PGE2, 4.5 ng/l for 6-keto-PGF1a and 9.0 ng/l for PGF2a and TxB2. The intra-assay variability was below 5.3%.

Calculations

The transport of the low-molecular-weight solutes was expressed as mass transfer area coefficients (MTAC). This is the maximal theoretical clearance of a solute transported from the blood to the dialysate by diffusion at time zero, before solute transport has started. The MTACs of urea, creatinine, urate, nitrate and cGMP were calculated according to the model of Waniek et al. [19], with a correction factor for plasma water [20]. An MTAC that exceeds the value expected on the basis of the molecular weight of a solute point to local i.p. release of the solute.

Peritoneal clearances of β2-microglobulin, albumin, IgG and α2-macroglubulin were calculated using the equation: Cl(μl/min) = (D × V)/(P × t), where Cl is the clearance, D is the dialysate concentration, V is the dialysate volume, P is the plasma concentration and t is the dwell time. These clearances and the free diffusion coefficients of the four proteins in water, were used to calculate the restriction coefficient. The restriction coefficient (RC) is the functional characterization of the intrinsic size-selective peritoneal permeability [6,21]. This parameter is the slope of the power relationship between the clearances of the above mentioned proteins (Cl) and their free diffusion coefficient in water (D20,ω) when plotted on a double logarithmic scale: Cl = constant × D20,ωRC.

The transport of fluid across the peritoneum was calculated as described previously by Imholz et al. [6]. Briefly, fluid transport from the peritoneal cavity is assumed to be by transcapillary back filtration and also by uptake into the lymphatic system. The effective lymphatic absorption rate can be approximated as the disappearance rate of dextrans 70 [22]. These calculations include all pathways of lymphatic drainage from the peritoneal cavity, both sub-diaphragmatic and interstitial. The transcapillary ultrafiltration was calculated from the dilution of dextran 70. The net ultrafiltration is the difference between the transcapillary ultrafiltration and the lymphatic absorption.

Results

Normal indomethacin dose

The dialysate with and without addition of indomethacin was well tolerated. The dialysate concentration of 6-keto-PGF1a decreased after addition of indomethacin from 93 ng/l (range 14–360 ng/l) to 8 ng/l (<5–12 ng/l), P = 0.006 (Figure 1). With indomethacin dialysate TxB2 decreased from 12 ng/l (range 9–23 ng/l) to 9 ng/l (<9–19 ng/l), P = 0.04 (Figure 1). The median decrease was 91% (range 78–94%) for 6-keto-PGF1a and 33% (range 0–71%) for TxB2. No difference was found in the dialysate concentration of the prostaglandin E2 after addition of indomethacin: control 69 ng/l (<18–111 ng/l), indomethacin 50 ng/l (<18–104 ng/l) (Figure 1). Six patients had a decrease in dialysate PGE2 concentrations. They were not different from the other four with regard to MTACs of low-molecular-weight solutes, protein clearances or the restriction coefficient to macromolecules (data not shown). The PGF2a concentration in dialysate was above the lower detection limit in four patients during the control experiment and was detectable in three patients after the addition of indomethacin: control range <9–14.5 ng/l, with indomethacin <9–21 ng/l.

The dialysate concentrations of nitrite were below the lower detection limit. The nitrate concentrations in the dialysate were 83.6 μmol/l (21.1–341.8 μmol/l) in the control dwell and 50.9 μmol/l (18.6–142.0 μmol/l) during the indomethacin dwell, P = 0.3. The cGMP concentrations in the dialysate were also not different: control 8 nmol/l (5.9–49.4 nmol/l) and with indomethacin 14.9 nmol/l (6.8–53.0 nmol/l), P = 0.3. Table 1 shows the MTACs of nitrate and cGMP in the control experiment and after the addition of indomethacin. No significant differences were found.

The MTACs of urea, creatinine and urate are summarized in Table 1. Only for urea a marginally greater MTAC was found with indomethacin. The MTAC values of creatinine and urate were similar with and without indomethacin. Also, no differences were found in the clearances of the proteins β2-microglobulin, albumin, IgG and α2-macroglubulin (Table 1). The restriction coefficient to macromolecules did not change.

The transcapillary ultrafiltration and the effective lymphatic absorption rate during the 4-h dwell were similar with and without indomethacin (Figure 2). Consequently, the net ultrafiltration profile, the difference between these two, did not change.

High indomethacin dose

Because no differences were found in PGE2 concentrations with the normal indomethacin dose, a higher dose during a longer time period was used in five other patients.

Table 1. MTACs of nitric oxide metabolized products, of urea, creatinine and urate, the peritoneal protein clearances and the restriction coefficient calculated on these protein clearances (see text for further explanation) with 1.36% glucose dialysate and with the normal dose of indomethacin. Values are given as medians with ranges

<table>
<thead>
<tr>
<th>MTAC (ml/min)</th>
<th>1.36% Glucose</th>
<th>+ Indomethacin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitrate</td>
<td>10.0 (4.6–12.8)</td>
<td>9.9 (4.1–12.1)</td>
<td>0.48</td>
</tr>
<tr>
<td>cGMP</td>
<td>8.7 (2.2–12.0)</td>
<td>8.0 (5.3–19.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>MTAC (ml/min)</td>
<td>urea</td>
<td>16.0 (12.1–23.2)</td>
<td>16.6 (13.8–23.6)</td>
</tr>
<tr>
<td>creatinine</td>
<td>9.5 (7.0–17.7)</td>
<td>10.2 (8.2–16.2)</td>
<td>0.22</td>
</tr>
<tr>
<td>urate</td>
<td>7.2 (4.9–14.9)</td>
<td>7.3 (5.8–13.7)</td>
<td>0.26</td>
</tr>
<tr>
<td>Clearance (μl/min)</td>
<td>β2-microglobulin</td>
<td>785 (599–2032)</td>
<td>911 (674–1799)</td>
</tr>
<tr>
<td>albumin</td>
<td>87 (50–176)</td>
<td>89 (58–164)</td>
<td>0.26</td>
</tr>
<tr>
<td>IgG</td>
<td>48 (17–98)</td>
<td>51 (26–88)</td>
<td>0.22</td>
</tr>
<tr>
<td>α2-macroglubulin</td>
<td>14 (5–38)</td>
<td>15 (6–27)</td>
<td>0.41</td>
</tr>
<tr>
<td>Restriction coefficient</td>
<td>2.43 (2.21–3.02)</td>
<td>2.48 (2.00–2.78)</td>
<td>0.29</td>
</tr>
</tbody>
</table>
patients. With indomethacin no difference was found in the dialysate concentration of PGE₂: control 69 ng/l (48–119) and indomethacin 63 ng/l (57–87). Also, no differences were found in the MTACs of urea, creatinine and urate and the peritoneal clearances of the proteins β2-microglobulin, albumin, IgG and α2-macroglobulin (Table 2). The restriction coefficients to macromolecules were similar and no effects were found on the peritoneal fluid kinetics (Table 2).

Discussion

The present study demonstrates that inhibition of the synthesis of 6-keto-PGF₁α and TxB₂ by i.p.-administered indomethacin had no effect on the peritoneal permeability characteristics during stable CAPD. Furthermore, no effect of indomethacin was found on

Table 2. MTACs of urea, creatinine and urate, the peritoneal protein clearances and the restriction coefficient calculated on these protein clearances (see text for further explanation) and the fluid kinetics in the high dose indomethacin experiments. Values are given as medians with ranges. None of the differences were significant

<table>
<thead>
<tr>
<th>MTAC (ml/min)</th>
<th>1.36% Glucose</th>
<th>+ High-dose indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>21.4 (16.0–27.5)</td>
<td>19.2 (14.9–20.7)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>12.4 (7.3–17.6)</td>
<td>10.4 (7.0–14.9)</td>
</tr>
<tr>
<td>Urate</td>
<td>9.4 (5.1–15.4)</td>
<td>7.9 (5.1–12.0)</td>
</tr>
<tr>
<td>Clearance (μl/min)</td>
<td>844 (704–1614)</td>
<td>966 (682–1326)</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>94 (64–149)</td>
<td>91 (58–132)</td>
</tr>
<tr>
<td>Albumin</td>
<td>53 (29–82)</td>
<td>48 (26–72)</td>
</tr>
<tr>
<td>IgG</td>
<td>18 (4–31)</td>
<td>14 (4–27)</td>
</tr>
<tr>
<td>Restriction coefficient</td>
<td>2.45 (2.11–2.96)</td>
<td>2.45 (2.13–2.90)</td>
</tr>
<tr>
<td>TCUFR (ml/min)</td>
<td>3.35 (2.45–5.31)</td>
<td>4.24 (3.08–5.81)</td>
</tr>
<tr>
<td>ELAR (ml/min)</td>
<td>1.29 (0.87–3.05)</td>
<td>1.62 (1.07–3.01)</td>
</tr>
<tr>
<td>NUFRI (ml/min)</td>
<td>1.62 (0.34–4.45)</td>
<td>2.53 (0.07–4.19)</td>
</tr>
</tbody>
</table>
parameters of nitric oxide synthesis, measured in this study.

Indomethacin inhibits cyclooxygenase activity and blocks the conversion of arachidonic acid to biologically active prostanooids [9]. Cyclooxygenase catalyses the conversion of PGG2 to PGH2. These endoperoxides have a short half-life time and are converted into biologically active prostaglandins and thromboxane A2. PGH2 is converted into the vasodilating PGE2 or into the vasoconstricting PGF2α. The biosynthesis of prostacyclin (PGI2) is catalysed by prostacyclin synthase, thromboxane synthase induces conversion of PGH2 into TXA2. PGI2 and TXA2 are rapidly degraded to respectively 6-keto-PGF1α and TXB2. These stable metabolites are usually measured as a representation of the active compounds.

The prostaglandins PGE2, 6-keto-PGF1α, PGE2 and TXB2 have been detected in the effluent of stable CAPD patients. Possible sources of prostanoids released into the dialysate are the mesothelial cells, macrophages, endothelial cells or fibroblasts. The mesothelial cells are capable to generate prostanoids in isolated peritoneum and in cell cultures [7,23,24]. Topley et al. [24] reported an increased prostanoid release by cultured human peritoneal mesothelial cells after incubation with supernatant from stimulated and unstimulated peritoneal macrophages. Peritoneal macrophages isolated from dialysate of CAPD patients during an infection free period were shown to secrete spontaneously the metabolites PGE2, 6-keto-PGF1α and TXB2 [25]. Also, endothelial cells and fibroblasts are able to produce prostaglandins [26–28].

In the present study the prostaglandin concentrations in the dialysate during the 4-h dwells were low, and sometimes even below the lower detection limit. However, the i.p. administration of 12.5 mg/l indomethacin resulted in a decrease in the dialysate concentrations of 6-keto-PGF1α and TXB2. The expected inhibition of PGE2 synthesis was not found. Also, with the high dose of indomethacin, no inhibition of PGE2 synthesis was found. Again, no differences were seen in the transport of small solutes, protein clearances or fluid kinetics. Therefore, the absence of inhibition of PGE2 synthesis is unlikely to be a result of a too low dose of indomethacin. Furthermore, with the dose of 12.5 mg/l we found marked inhibition of prostaglandin synthesis during peritonitis, a situation with increased prostaglandin release [13]. We analysed this in more detail by comparing the dialysate concentrations of the prostaglandins reported by Zemel et al. [13] in peritonitis during indomethacin, with those after recovery from the infection. The dialysate concentrations of 6-keto-PGF1α and TXB2 in that study were significantly lower during indomethacin treatment than control values after recovery. However, this difference was not present for the dialysate concentrations of PGE2, which suggests that indomethacin is only able to inhibit augmented synthesis of PGE2. One other study about i.p. cyclooxygenase inhibition in stable CAPD patients has been published. Steinhauser and Schollmeyer [10] studied four CAPD patients after recovery of peritonitis and found that i.p. administration of 12.5 mg/l indomethacin for 24 h only diminished the peritoneal generation of PGE2, while no effect was observed on the synthesis of other prostanooids and the peritoneal protein loss. These findings are difficult to interpret because of the small number of patients.

CAPD-related peritonitis is a situation with increased dialysate concentrations of various inflammatory mediators, including prostaglandins [10,11,29]. It is accompanied by increases in the transport of solutes, pointing to a larger effective surface area and increased intrinsic peritoneal permeability [11,30]. In previous studies in CAPD patients during peritonitis, evidence was obtained that changes in dialysate PGE2 concentrations were related to changes in intrinsic permeability, but not to changes in the effective surface area [11]. This was investigated both with a statistical method [11] and also by PGE2 synthesis inhibition using i.p. indomethacin [13]. These findings are in accordance with the present study where no inhibition of PGE2 synthesis resulted in no changes in the intrinsic permeability. An absence of the effect of intraperitoneal indomethacin on the intrinsic permeability in stable uninfected CAPD patients may either mean that these prostaglandins are only involved in the regulation of vascular permeability during pathologi- cal conditions, or may be caused by insufficient inhibition of PGE2. However, the inability of a high dose of indomethacin to inhibit PGE2 suggests that PGE2 synthesis during stable CAPD is at a baseline level. This supports the first mentioned possibility.

Nitric oxide may be involved in the release of PGE2, possibly by direct activation of cyclooxygenase [31]. We therefore investigated the effect of indomethacin on parameters of nitric oxide activity, such as nitrate and the mediator cyclic guanosine monophosphate (cGMP).

We found no evidence in the present study that indomethacin had any effect on the nitric oxide system: dialysate concentrations of nitrate were below the detection limit and the MTACs for nitrate and cGMP concentrations were not affected by indomethacin.

It is concluded that during stable CAPD, one i.p. administration of indomethacin during 4-h dwells caused inhibition of the synthesis of 6-keto-PGF1α and TXB2. However, a decrease in the release of these prostaglandins did not result in alterations in the effective peritoneal surface area, the intrinsic permeability to macromolecules and the fluid kinetics. This is consistent with our previous reports during peritonitis indicating that 6-keto-PGF1α and TXB2 are not related to peritoneal transport kinetics. Although, theoretically, the indomethacin induced decrease in the vasodilating 6-keto-PGF1α could be counteracted by the decrease in the vasoconstricting TXB2, we think this possibility less likely because the decrease in 6-keto-PGF1α was more pronounced than that in TXB2, and because in general the effects of non-steroidal anti-inflammatory drugs are more pronounced on the vasodilating than on the vasoconstricting prostaglandins. PGE2 was related to intrinsic permeability during
peritonitis, but this prostaglandin could not be inhibited by indomethacin in the present study, neither using a normal indomethacin dose, nor using a high dose. Also, no differences were found in parameters for nitric oxide synthesis. Therefore, 6-keto-PGF1α and TxB2 are not likely to be involved in the regulation of peritoneal transport during stable CAPD, while an effect of PGE2 on the size-selectivity of the peritoneum is probably only present during acute peritonitis.

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