Absence of gadolinium deposits in the peritoneal membrane of patients with encapsulating peritoneal sclerosis

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Abstract

Background. Encapsulating peritoneal sclerosis (EPS) is a severe complication of long-term peritoneal dialysis (PD) characterized by the development of an extensive fibrosis of the visceral peritoneum that may eventually lead to intestinal constriction. Its cause remains elusive. Nephrogenic systemic fibrosis (NSF), a disabling disease that can follow gadolinium-based contrast injection during magnetic resonance imaging, is characterized by systemic fibrosis of the skin, joints, liver, heart and vessels. Affected tissues are infiltrated by CD34+ and CD68+ fibroblasts. In the present study, we tested the hypothesis that EPS could have been triggered by a previous gadolinium injection.

Methods. We performed histopathological analysis of the peritoneal membrane of two EPS and two control patients all exposed to long-term PD, including immunostaining with CD34 and CD68. The presence of gadolinium and other metals was also assessed by conventional and energy-filtered transmission electron microscopy.

Results. Numerous CD34+ and CD68+ cells were found in both the EPS and control patients within the vascular endothelium and in macrophages, respectively, but not in interstitial fibrocytes, as it could be expected in NSF. No trace of gadolinium deposits could be found in the four peritoneal samples; disperse tiny iron inclusions were evidenced in the connective tissue of both EPS patients.

Conclusions. These findings argue against the implication of gadolinium in the development of EPS in long-term PD patients.

Keywords: encapsulating peritoneal sclerosis; gadolinium; magnetic resonance imaging; nephrogenic systemic fibrosis; peritoneal dialysis

Introduction

Encapsulating peritoneal sclerosis (EPS) is a rare but devastating complication of long-term peritoneal dialysis (PD). It is characterized by fibrosis of the visceral peritoneum that may eventually lead to extensive intestinal constriction with persistent, intermittent or recurrent bowel obstruction, sometimes associated with bloody effluent. Weight loss and malnutrition usually ensue. Mortality is high, despite various treatment options [1–3]. The cause of EPS remains elusive, though multiple triggering factors have been incriminated (long-term exposure of the peritoneal membrane to poorly biocompatible peritoneal dialysates, repeated peritoneal infections, genetic predispositions…) [1,4]. An increased incidence of EPS following renal transplantation (TP) in PD patients has been suspected in the last 2–3 years [5–7].

Recently, a systemic illness characterized by skin and joint fibrosis [8], together with systemic inflammation [9–11], has been reported in end-stage renal disease and haemodialysis patients and has been linked to a previous exposition to gadolinium-based contrast material during magnetic resonance imaging (MRI) [9,10]. The disorder has been called nephrogenic systemic fibrosis (NSF). Gadolinium, initially demonstrated in the skin of NSF patients [12–14], was subsequently found in numerous other tissues including the myocardium, blood vessels, skeletal muscles, testis, liver and lungs [14–17]. In patients with chronic renal disease, the clearance of gadolinium is markedly delayed, allowing a process of transmetalation. During this process, ions such as calcium, zinc, iron, copper and aluminium may substitute for gadolinium in the metal complex, leading to deposition of free gadolinium and iron in tissues affected by NSF [15,18]. It has been hypothesized that this massive free gadolinium and iron deposition...
induces the pro-inflammatory process that leads to NSF. The diagnosis of NSF relies on the demonstration within a deep dermal biopsy of fibrosis and specific histopathologic features, such as the identification of CD34+ dendritic and CD68+ mononucleated cells among collagen fibres [19,20]. Gadolinium is poorly removed by conventional PD [21] and NSF has also been described in PD patients [22–24].

In the present study, we tested the hypothesis that gadolinium could deposit within the peritoneal tissue of PD patients with EPS, thus representing a localized form of NSF confined to the peritoneal membrane, a hypothesis that might explain the recently observed increased incidence of post-renal transplant EPS. Peritoneal biopsies from two EPS patients and one control patient exposed to gadolinium while on PD and from one control PD patient non-exposed to gadolinium were obtained after renal TP and studied by immunostaining and ultrastructural analysis.

Materials and methods

Clinical presentation

Patient 1. A 61-year-old male with end-stage renal failure (ESRF) secondary to membranoproliferative glomerulonephritis received a renal cadaver TP after 100 months on PD. He had not presented any infectious peritonitis while on PD. MRI of the aortoiliac vessels with gadolinium injection (Omniscan®; a single dose of 10mmol) had been performed in May 2005, 9 months before TP. Immunosuppressive regimen included tacrolimus, azathioprine and steroids. A peritoneal biopsy, taken at the time of peritoneal catheter removal, 2 months after TP, showed a denuded mesothelium and a thickened, highly vascularized submesothelial fibrous band, suggestive of EPS. Four months later, he presented with intestinal obstruction. At surgery, the macroscopic appearance of the peritoneal membrane confirmed the diagnosis of EPS so that no specific intervention was performed. The symptomatology resolved with nasogastric aspiration, fasting and temporary parenteral nutrition. The steroid dosage was transiently increased and tamoxifen was initiated. Subsequently, the patient was re-admitted four times for similar episodes, all resolving without surgery. Currently, 30 months after TP, the patient is asymptomatic, though encasement of small-bowel loops and thickening of the intestinal wall are still present (Figure 1). No systemic signs of NSF appeared within his follow-up.

Patient 2. A 67-year-old male with ESRF secondary to nephrosclerosis received a renal cadaver TP after 55 months on PD. He had presented a single episode of Staphylococcus epidermidis peritonitis after 7 months on PD. MRI of the aortoiliac vessels with gadolinium injection (Omniscan®, a single dose of 10mmol) had been performed in February 2005, 20 months before TP. Immunosuppressive regimen included cyclosporine, azathioprine and steroids. One week after TP, he underwent an aortobifemoral replacement because of spontaneous iliac artery dissection. The intervention was complicated by Serratia marcescens peritonitis that has been cured with meropenem. A peritoneal biopsy, taken at the time of PD catheter removal, 5 weeks after TP, showed a denuded mesothelium and a thickened, highly vascularized submesothelial fibrous band, suggestive of EPS. Eight months after TP, he presented with several bouts of intestinal obstruction. Abdominal CT scan disclosed peritoneal thickening and several loculated fluid collections, typical of EPS. Total parenteral nutrition was initiated, together with an increase in the steroids dosage and the prescription of tamoxifen, without improvement of the clinical condition. One year later, enterolysis with 30 cm small-bowel resection was performed. The symptomatology resolved with nasogastric aspiration, fasting and temporary parenteral nutrition. The steroid dosage was transferred using the electron energy zero-loss mode, all uranyl acetate and lead citrate and examined under the LEO912AB (Carl Zeiss, Oberkochen) energy-filtered transmission electron microscope (EFTEM). Images were acquired by a 1 × 1-k pixel side-entry digital camera. The elemental analysis of gadolinium, calcium and iron was performed – 31 for element — 144.

Histopathology

All peritoneal biopsies taken in the parietal peritoneum at the time of catheter removal from both EPS and control patients were fixed in 4% buffered formalin (pH 7.0). Paraffin sections were stained with haematoxylin and eosin (H&E) for light microscopy and processed by immunohistochemistry for the assessment of the cellular expression of CD34 and CD68.

Conventional electron microscopy and electron spectroscopic imaging

From a selected (positive CD34 and CD 68 evidence) paraffin block with embedded peritoneal tissue (one per patient) H&E slide guided tissue cores (three per block, diameter 2mm) were punched, deparaffinized in xylene, rehydrated with downgraded ethanols and cacodylate buffer and post-fixed with 4% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) and subsequently with osmium tetroxide (both 1h). After being milled into smaller pieces, this tissue was routinely dehydrated in an automated manner in graded ethanols (LYNX, Leica/Vienna tissue processor) and embedded in epoxy resins (EmBed 812, Science Services/Munich). Double-stained (toluidine blue/basic fuchsin) semi-thin sections were prepared for relevant structure trimming for the adjacent ultramicrotomy (Ultracut S, Leica/Vienna)—two (control tissue) or three (EPS tissue) Epon blocks per punched tissue core. From the EPS patient blocks, an additional step (100µm deeper) of semi-thin and following ultrathin sections were prepared. For conventional electron microscopy examination, the produced ultrathin sections (80nm) were double stained with aqueous uranyl acetate and lead citrate and examined under the LEOS912AB (Carl Zeiss, Oberkochen) energy-filtered transmission electron microscope (EFTEM), operated at 100kV in electron energy zero-loss mode, all images were acquired by a 1 × 1-k pixel side-entry digital camera.

The elemental analysis of gadolinium, calcium and iron was performed on very thin sections (approximately 40nm) without any heavy metal post-staining using the electron energy-filtered microscope operation mode (EFTEM).

This technique provides a very high structural resolution (0.1nm) [25] and sensitivity (three to six atoms, element dependent) [26–31] for element detection and localization. It is based on the phenomenon that primary beam electrons passing through a sample interact with target atoms.
of the specimen and lose a defined, element-specific amount of energy (‘inelastic scattering’). The energy loss of the beam electrons is analysed by an in-column integrated energy filter (spectrometer); a special slit aperture selects electrons at the element-specific energy-loss level for imaging the elemental distribution in the sample.

In resin-embedded tissue specimens, two modes of EFTEM are used: electron energy-loss spectroscopy (EELS) records the whole energy-loss range (0–2,500 eV) as a complete energy spectrum where ‘edges’ at characteristic energy levels provide information about the chemical composition of the sample. The spatial distribution of elements present in the sample can be mapped by electron spectroscopic imaging (ESI) using exclusively inelastically scattered electrons with the element-specific energy loss [32–38].

More technical details of the applied ESI and EELS are described elsewhere [14].

Results

Histopathology and immunostaining

Representative histologic changes of the parietal peritoneal membrane of Patient 1 are shown in Figure 2. H&E-stained sections of the peritoneal tissue showed a denuded mesothelium and a thickened, highly vascularized submesothelial fibrous band together with normal adjacent adipose tissue. The upper part of the submesothelial fibrous band had a fibrohyalin appearance with low inflammatory cellular infiltration (Figure 2A). Immunoperoxidase staining of the peritoneal wall showed arteriolar endothelial cells expressing CD34 (Figure 2B) and several macrophages expressing CD68 (Figure 2C). Neither of the markers were expressed by peritoneal fibrocytes. Similar findings were observed on the peritoneal biopsy of Patient 2.

H&E-stained sections of the peritoneal tissue of both control patients showed a well-preserved peritoneal membrane (Figure 2D). Immunoperoxidase staining showed arteriolar endothelial cells expressing CD34 (Figure 2E) and numerous macrophages expressing CD68 (Figure 2F).

Conventional electron microscopy and ESI

Electron microscopy analysis of Patients 1 and 2 peritoneal tissues showed abundant collagen and scattered elastic fibres. There were also some electron-optically dark granular deposits recognized as mast cell granules and artificially condensed chromatin. In both patients, very tiny (0.1–2.0 μm, mostly approximately 0.3 μm diameter) singular/multiple electron-optically dark inclusions were found in cell protrusions (probable macrophages) disseminated in the connective tissue. The ESI and EELS spectral analysis of those inclusions was negative for gadolinium and calcium, but positive for iron in some of them (Figure 3A–C). Peritoneal tissues from both controls only showed moderate fibrotic tissue with blood vessels.
Discussion

The absence of clinical signs of NSF in both our EPS patients (despite the exposure to linear chelates of gadolinium, i.e. those with the highest risk of toxicity) [4,18], together with the peritoneal biopsy examinations that showed a highly fibrotic process without CD34 and CD68 immunostaining in peritoneal fibrocytes or electron microscopic gadolinium deposits, argue against the implication of gadolinium in the EPS development of both our PD patients.

The diagnosis of NSF in the skin relies on histopathologic signs of fibrosis together with the presence in the derma of fibrocytes and histiocytic cells expressing CD34 (a marker of endothelial cells) and CD68 (a marker of macrophages) antigens, respectively. We looked for the presence of those cells within the fibrous peritoneal tissue of two EPS patients in comparison with two control PD patients without signs of EPS. We found a similar strong expression of CD34 within the peritoneum of both EPS and control patients, not by fibrocytes as it can be seen in NSF patients, but by the numerous peritoneal capillaries. This latter observation reflects the neoangiogenesis process commonly associated with long-term PD [39]. Likewise, a virtually not different strong expression of CD68 by numerous resident interstitial macrophages was found in both EPS and control peritoneal membranes, which is a common finding in the peritoneal membrane of long-term PD patients [40].

In patients with NSF, the pivotal role of gadolinium was supported by the demonstration of the presence of gadolinium and of iron chelates within the derma using ESI and EELS analyses [14].

In addition, tissue fibrosis associated with the deposition of gadolinium chelates has been demonstrated in numerous tissues, including the liver, the heart and the vessels [16,17]. In the present study, we could not detect any gadolinium chelates within the peritoneal membrane.

Fig. 3. ESI and parallel EELS: (A) iron mapping on electron-optically dark inclusions found in cell protrusions (probable macrophages) disseminated in the peritoneal connective tissue at high magnification (original magnification ×10 000), the net iron signal image is superimposed on the inverted HCI image showing the precise localization of the signal as a red spot on the tissue structure; (B) parallel EELS spectrum [the confirmation of a particular element is demonstrated by its defined energy-loss edge at the energy level necessary for atom inner shell ionization; record of the measured gadolinium signal (red) does not correspond to the reference gadolinium spectrum (green), allowing to exclude the presence of gadolinium in the peritoneal tissue]; (C) parallel EELS spectrum [record of the measured iron signal (red) corresponds to the reference iron spectrum (green), allowing to document the presence of iron deposits in the peritoneal tissue].
of both our EPS and control patients (despite numerous samples and step sections examined). This latter point is probably the key element to innocent gadolinium in the development of EPS. A word of caution has, however, to be raised since some iron-positive signals have been found in the peritoneal tissues of both patients; a finding that has also been seen in association with gadolinium deposits [14,16]. A contamination of the present samples by iron-containing microtome blade can be excluded by the use of a diamond knife and by strict contact avoidance between the samples with iron-containing surfaces [14]. Though a transmetallation phenomenon cannot be entirely ruled out [18,41], iron load, due to multiple IV iron infusions to raise haemoglobin level, as frequently observed in dialysis patients, is more likely to explain this finding [42,43]. Also, the peritoneal biopsies have been taken in the parietal peritoneum; we, however, do not think that the visceral peritoneum might have shown different results.

The lag time between NSF development and gadolinium injection is usually below 3 months according to both Markmann [10] and Swaminathan [16]. In both patients with EPS from the present study, the delay between gadolinium injection and EPS diagnosis was 9 and 20 months, respectively. It seems thus unlikely that gadolinium could have triggered the fibrotic process of EPS and have subsequently disappeared totally from the peritoneal tissues, given its high tissular affinity and long half-life. We, however, have to acknowledge that the remoteness of the gadolinium exposure in our patients and the limited peritoneal samplings represent the main limitations of the present study. Still, Schroeder et al. recently found persisting dermal gadolinium deposits in NSF patients more than 3 years after the last gadolinium administration [14].

Finally, the absence of gadolinium exposure in one patient from our recent post-renal TP EPS series [6] further supports the absence of link between EPS and gadolinium.

Conclusion

The present observation implies that a search for another aetio-pathogenic factor than gadolinium injection should be pursued in EPS following PD. The risk of EPS development after PD therapy is, therefore, not yet behind us with the avoidance of gadolinium injection in PD patients.

Conflicts of interest statement. None declared.

References


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