The pathogenesis of pulmonary hypertension in haemodialysis patients via arterio-venous access

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Abstract

Background. We recently have shown a high incidence of unexplained pulmonary hypertension (PHT) in end-stage renal disease (ESRD) patients on chronic haemodialysis (HD) therapy via arterio-venous (A-V) access. This study evaluated the possibility that PHT in these patients is triggered or aggravated by chronic HD via surgical A-V access, and the role of endothelin-1 (ET-1) and nitric oxide (NO) in this syndrome.

Methods. Forty-two HD patients underwent clinical evaluation. Pulmonary artery pressure (PAP) was evaluated using Doppler echocardiography. Levels of ET-1 and NO metabolites in plasma were determined before and after the HD procedure and were compared between subgroups of patients with and without PHT.

Results. Out of 42 HD patients studied, 20 patients (48%) had PHT (PAP = 46 ± 2; range 36–82 mmHg) while the rest had a normal PAP (29 ± 1 mmHg) (P < 0.0001). HD patients with PHT had higher cardiac output compared with those with normal PAP (6.0 ± 1.2 vs 5.2 ± 0.9 l/min, P < 0.034). HD patients, with or without PHT, had elevated plasma ET-1 levels compared with controls (1.6 ± 0.7 and 2.4 ± 0.8 fmol/ml vs 1.0 ± 0.2, P < 0.05) that remained unchanged after the HD procedure. HD patients without PHT and control subjects showed similar basal plasma levels of NO2 + NO3 (24.2 ± 5.2 vs 19.7 ± 3.1 μM, P > 0.05) that was significantly higher compared with HD patients with PHT (14.3 ± 2.3 μM, P < 0.05). HD therapy caused a significant increase in plasma NO metabolites that was greater in patients without PHT (from 24.2 ± 5.2 to 77.1 ± 9.6 μM, P < 0.0001, and from 14.3 ± 2.3 to 39.9 ± 11.4 μM, P < 0.0074, respectively). Significant declines in PAP (from 49.8 ± 2.8 to 38.6 ± 2.2 mmHg, P < 0.004) and cardiac output (CO) (from 7.6 ± 0.6 to 6.1 ± 0.31/min, P < 0.03) were found in 11 HD patients with PHT that underwent successful transplantation. Similarly, temporary closure of the A-V access by a sphygmomanometer in eight patients with PHT resulted in a transient decrease in CO (from 6.4 ± 0.6 to 5.3 ± 0.51/min, P = 0.18) and systolic PAP (from 47.2 ± 3.8 to 34.6 ± 2.8 mmHg, P < 0.028).

Conclusions. This study demonstrates a high prevalence of PHT among patients with ESRD on chronic HD via a surgical A-V fistula. In view of the vasodilatory and antimitogenic properties of NO, it is possible that the attenuated basal and HD-induced NO production in patients with PHT contributes to the increased pulmonary vascular tone. Furthermore, the partial restoration of normal PAP and CO in HD patients that underwent either temporal A-V shunt closure or successful transplantation indicates that excessive pulmonary blood flow is involved in the pathogenesis of the disease.

Keywords: arterio-venous access; endothelin; end-stage renal disease; haemodialysis; nitric oxide; pulmonary hypertension

Introduction

Pulmonary hypertension (PHT) is an elevation of pulmonary arterial pressure (PAP) that can be the result of heart, lung or systemic disorders [1,2]. Regardless of the aetiology, the morbidity and the mortality from long-standing PHT exceed that expected from the causative condition. PHT involves vasoconstriction and obliteration of the lumen of small vessels in the lungs by plexiform lesions resulting in increased resistance to flow [3]. Proposed mechanisms for the formation of the plexiform lesion include dysregulation of endothelial growth and angiogenic response to local triggers [4].
We have recently shown a 40% incidence of PHT as detected by Doppler echocardiography in patients with end-stage renal disease (ESRD) on chronic haemodialysis (HD) therapy via arterial–venous (A-V) access [5]. Affected patients had significantly higher cardiac output (CO). Temporary A-V access closure and successful kidney transplantation caused a significant fall in PAP values to a normal range. Based on these data, we presumed that the pulmonary circulation of some uraemic patients on chronic HD therapy is in a vasoconstriction state. Most probably, the A-V access-induced high CO contributes to the development of provoked PHT.

Local vascular tone and function are regulated by the balance between vasodilators, such as prostacyclin and nitric oxide (NO), and vasoconstrictors, such as thromboxane A2 and endothelin-1 (ET-1) [1,4]. The current study was carried out to investigate the possibility that PHT in these patients is triggered/aggravated by chronic HD via a surgical A-V access, and to explore the involvement of the ET-1 and NO systems in the pathogenesis of PHT in HD patients.

Patients and methods

Patients

Our institutional clinical research ethics review board approved the research protocol and informed consent was obtained from all participants. Twenty-eight patients from the initial study population (of 58 ESRD patients on chronic HD therapy via A-V access) were excluded from this study, due to known cardiac, pulmonary and systemic diseases. Five patients underwent kidney transplantation, nine patients died during the follow-up and 14 patients refused to participate in the study. Twelve additional patients, not included in the original study, increased the study population to 42 eligible patients without known cause of PHT. The control group consisted of 20 normal volunteers, all without known cause for PHT.

Patient evaluation

Systolic PAP was estimated in the 42 HD patients by Gladwin et al. [6]. To avoid overestimation of PAP due to volume overload, the echo studies in HD patients were performed within 1 h after completion of HD, when the patients were at optimal dry weight according to hydration status, blood pressure and weight.

An experienced operator (S.A.R.) performed all the echocardiographic studies, using an Acuson Sequoia, Aspen or I28 XP (Mountain View, CA) ultrasound machine. A complete two-dimensional, M-mode and Doppler echocardiographic study was obtained from each patient. A tricuspid regurgitation systolic jet was recorded from the parasternal or apical window with the continuous-wave Doppler probe. Systolic right ventricular (or pulmonary artery) pressure was calculated using the modified Bernoulli equation: \( PAP = 4 \times (\text{tricuspid systolic jet}^2) + 10 \text{mmHg} \) (estimated right atrial pressure). The accuracy of systolic PAP estimation by Doppler in our laboratory was previously published [7]. PHT was defined as a systolic PAP ≥35 mmHg. Stroke volume was estimated from the left ventricular outflow tract velocity time integral×diameter, and CO was calculated by multiplying the stroke volume by the heart rate.

The general data concerning patients (age, sex, co-morbidities and medications used) and kidney disease (etiolog of renal failure, age at onset, duration of HD therapy and access location, brachial or radial) were recorded directly from the patients or from their hospital files. Blood tests for haemoglobin, haematocrit, calcium, phosphorus and parathyroid hormone level were sampled at the end of the HD therapy within 1 week from the echocardiography study. For measures sampled frequently (haemoglobin, haematocrit, calcium and phosphorus), the mean values from the 6 months preceding the echo study were presented. Plasma levels of intact PTH were determined by two site chemoluminescent enzyme-labeled immunometric assay (DPC, LA, CA, USA).

Patients with PHT >35 mmHg were evaluated further by an experienced pulmonologist (M.Y.) with the intention to disclose other causes of PHT. They underwent chest X-radiography and computed tomography, complete pulmonary function tests, measurement of blood gases and \( \text{O}_2 \) saturation, and ventilation–perfusion lung scan as previously described [1,5].

We also examined the effect of A-V access compression with a sphygmomanometer for 1 min (\( n = 8 \)) and the effect of successful kidney transplantation (\( n = 11 \)) on PAP and CO values in HD patients with PHT. The corresponding PAP and CO values were compared before and after the A-V closure for 1 min, or pre- and post-kidney transplantation. Time intervals between renal transplantation and measurement of PAP and CO range between 11 months and 4 years.

Determination of ET-1

Plasma ET-1 levels were determined in 42 patients on chronic HD, with either normal pulmonary blood pressure (PAP <35 mmHg) or PHT (PAP >35 mmHg) by enzyme-linked immunosorbent assay (ELISA; Biomedica, Vienna, Austria). All the blood samples were collected into pre-cooled tubes containing potassium EDTA and the protease inhibitor aprotinin, before and after the HD procedure. Plasma separated immediately in a cold centrifuge at 3000 r.p.m. and samples were stored at −70°C until analysis was performed in a single assay. Twenty matched healthy volunteer subjects served as controls. The ET-1 antibody employed in this assay has a 100% cross-reactivity with ET-2, and <5% and <1% cross-reaction with ET-3 and Big ET-1, respectively.
**Determination of NO₂ + NO₃**

NO metabolites (NO₂ and NO₃) in plasma of the patients and volunteers described above were determined before and after the dialysis procedure with the Griess method using a commercial kit. This kit is a colorimetric assay applying nitrate reductase, which converts nitrate to nitrite, and total nitrite was measured at 540 nm absorbance by reaction with Griess reagent (sulfanilamide and naphthalene-ethylene diamine dihydrochloride). Amounts of nitrite in the plasma were estimated by a standard curve obtained from enzymatic conversion of NaNO₃ to nitrite. Twenty matched healthy volunteer subjects served as controls for determining the basal circulatory levels of ET-1 and NO metabolites.

**Data analysis and statistics**

Clinical variables were compared between HD patients with and without PHT by using analysis of variance (ANOVA), followed by t-test. The correlation between categorical variables, such as PHT and survival from onset of HD therapy, was investigated by the χ² test. Values are expressed as the mean ± SEM. P-values of <0.05 were considered as significant.

**Results**

Data of the study patients (n = 42), 27 men, 15 women, mean age of 60.8 ± 11.8 years, range 31–81, receiving HD therapy are summarized in Table 1. Mean duration of HD therapy was 43 ± 29 months, range 6–120. The common aetiologies of renal failure were diabetes mellitus, arterial hypertension and glomerulopathy. Twenty patients (48%) had PHT.

Data from the 20 HD patients with PHT were compared with those of the 22 HD patients without PHT (Table 2). The CO was significantly higher among the PHT subgroup (5.95 ± 1.2 vs 5.2 ± 0.91 l/min, P < 0.034) (Table 2). The height and weight were not significantly different between both subgroups. Similarly, the haemoglobin and haematocrit levels did not differ significantly between patients with and without PHT (10.6 ± 1.6 vs 11.2 ± 1.7 and 33.3 ± 5.4 vs 35.7 ± 5.1, respectively). The incidence of antihypertensive medications and the distribution of these agents did not differ significantly between the two subgroups. The mean duration of HD therapy was not significantly lower in the PHT subgroup (35 ± 25 vs 50 ± 30 months). Other variables, such as anatomic location of the dialysis vascular access, parathyroid hormone levels and calcium–phosphorus product, did not differ significantly between the examined subgroups. Most patients (70–73%) from both subgroups (with and without PHT) tolerated the HD procedure and did not develop severe hypotension. However, six patients in each subgroup were classified as classic dialysis intolerant. No correlation was found between the incidence of HD intolerance and plasma NO levels.

**Table 1. Data on 42 patients with ESRD on HD via surgical A-V access**

| Age (years) | Mean age ±SEM | 60.8 ± 11.8 |
| Range | 31–81 |

| Sex | Male/female | 27/15 |
| M/F ratio | 1.8 |

| Aetiology of renal failure | Diabetes mellitus | 15 |
| Hypertension | 12 |
| Glomerulonephritis | 6 |
| Chronic pyelonephritis | 4 |
| Nephrolithiasis | 3 |
| Unknown | 2 |

| Duration of dialysis (months) | Mean | 43 ± 29 |
| Range | 6–120 |

| Follow-up period from echo (months) | Mean | 34 ± 35 |
| Range | 21–65 |

| Outcome | Died | 7 (16.6%) |
| Alive | 35 |

Seven (16.6%) patients died during follow-up, four with and three without PHT, providing mortality rates of 20 and 14%, respectively, P = NS.

**Plasma ET-1 levels**

As shown in Figure 1A, plasma concentrations of ET-1 in HD patients (combined with and without PHT) were significantly higher than those obtained in normal subjects (2.13 ± 0.37 vs 0.99 ± 0.17 fmol/ml, P < 0.05). Plasma levels of ET-1 were not changed by the dialysis procedure. The plasma levels of ET-1 did not differ significantly between HD patients with and without PHT (1.60 ± 0.67 vs 2.35 ± 0.77 fmol/ml before HD and 1.58 ± 0.68 vs 2.43 ± 0.79 fmol/ml after HD) (Figure 1B).

**Plasma NO metabolite levels**

As shown in Figure 2A, pre-HD plasma concentrations of NO₂ + NO₃ in the study population were similar to those obtained in normal subjects (19.73 ± 3.13 vs 22.30 ± 3.80 µM). However, following the HD procedure, plasma levels of NO₂ + NO₃ increased from 19.73 ± 3.13 to 60.29 ± 8.00 µM (P < 0.001). Compared with HD without PHT, patients with PHT had lower pre-HD circulatory levels of NO metabolites (14.34 ± 2.32 vs 24.17 ± 5.21 µM, P < 0.05). Interestingly, following the HD procedure, plasma NO₂ + NO₃ levels of both subgroups of HD patients increased significantly (Figure 2B). However, this increase was more remarkable in patients without PHT (from 24.17 ± 5.21 to 77.10 ± 9.60 µM, P < 0.0001) compared with PHT patients (from 14.34 ± 2.32 to 39.87 ± 11.41 µM, P < 0.0074).
Kidney transplantation

Eleven HD patients with PHT underwent successful kidney transplantation during the study period. The pre-transplantation mean systolic PAP of 49.8 ± 2.8 mmHg (range, 37–65) decreased to 38.6 ± 2.2 mmHg (range, 28–50), P < 0.004 (Figure 3A). The observed reduction in PAP following transplantation was associated with a significant decline in CO from 7.6 ± 0.6 to 6.1 ± 0.3 l/min, P < 0.03, (Figure 3B). The reduction in PAP following kidney transplantation was observed whether the A-V fistula was closed (PAP decreased from 47 ± 2.4 to 37.1 ± 1.7 mmHg, P < 0.014, n = 7) or opened (PAP decreased from 54.8 ± 6.1 to 40.8 ± 5.1 mmHg, P = 0.13, n = 4). Similarly, CO was reduced in transplanted patients with both closed A-V fistula (from 8.1 ± 0.92 to 6.0 ± 0.35 l/min, P < 0.05) and opened fistula (from 6.8 ± 0.74 to 5.9 ± 0.20 l/min, P = 0.27).

A-V access compression

PAP and CO were measured in eight HD patients with significant PHT before and 1 min after A-V access compression. During this manoeuvre, the systolic PAP decreased from 47.2 ± 3.8 to 34.6 ± 2.8 mmHg, P < 0.028 (Figure 3C), while the CO decreased from 6.4 ± 0.6 to 5.3 ± 0.5 l/min, P = 0.18 (Figure 3D).

Discussion

PHT is defined as a sustained elevation of PAP to >25 mmHg at rest or to >30 mmHg with exercise. The ability of the pulmonary circulation to dilate and recruit unused blood vessels prevents development of PHT in conditions characterized by increased pulmonary blood flow [3]. Pulmonary hypertensive
disease in HD patients is a relatively new entity in which elevated CO is an important factor. Previously, we reported that PAP increased significantly following initiation of HD therapy via A-V access and decreased significantly after successful kidney transplantation, as well as after short A-V access compression, indicating that both ESRD and A-V access contribute to the pathogenesis of PHT. However, the fact that only about half of the uraemic patients developed PHT suggests that mechanisms other than uraemia may be involved in this disorder. These may include underlying concomitant diseases such as diabetes mellitus or systemic hypertension, although the percentages of diabetic or hypertensive patients in both subgroups of patients with and without PHT were identical.

This study investigated the role of two endothelial-derived molecules, ET and NO, in the pathogenesis of this syndrome. Diminished vasodilatation response to the A-V access-induced elevated CO is a possible explanation for the elevated PAP in some uraemic patients. Endothelial dysfunction that has been described in PHT [8,9] and in uraemia [10] is a proposed mechanism.

In line with our previous findings that were published 2 years ago [5], 48% of the patients had PHT, with significantly elevated CO. The present findings indicate that affected patients had diminished NO production while their ET system remained intact.

Based on the data presented, we may conclude that the pulmonary hypertensive disease of HD patients is a unique form of PHT in which elevated CO and uraemic-induced endothelial dysfunction exist. This endothelial dysfunction manifests as reduced NO production, augments the tonus of the pulmonary vascular, reduces the capacity of the pulmonary circulation to maintain the A-V access-mediated elevated CO and subsequently causes PHT.

NO is a gaseous molecule that is produced in the endothelial cells and is involved in the regulation of tonus in both the pulmonary and systemic circulation [11]. Normally, the pulmonary endothelial cells increase the synthesis of NO in response to increased pulmonary blood flow and pressure in an attempt to restore normal vascular tone [12]. Reduced expression of endothelial NO synthase in the lungs of patients with PHT suggests that reduced production of this molecule may be involved in the pathogenesis of PHT [13]. Impaired NO production and reduced sensitivity to NO have been described in patients with chronic renal failure [14], but the impact of this fault on the pulmonary circulation in uraemia has not been studied previously. So far, endothelial dysfunction in uraemia has been related to the pathogenesis of hypertension and to accelerated atherosclerosis. This study demonstrates, for the first time, a link between impaired NO production and PHT in uraemic patients receiving chronic HD therapy via A-V access. Previous studies demonstrated that NO production is enhanced in HD patients following the HD procedure. This effect is attributed to the biocompatibility of the dialyser. The mechanisms that interfere with NO activity in uraemia are still not clear. Occurrence of endothelial dysfunction in all spectra of chronic renal failure suggests that uraemia may directly promote this fault. Reduced NO bio-availability of the NO substrate L-arginine, reduced expression of NO synthase in the relevant organs, interaction of NO with reactive oxygen species and accumulation of endogenous inhibitors of NO synthase, such as asymmetric dimethylarginine and homocysteine, are all proposed mechanisms [10,15].

Changes in shear stress can induce patterns of gene expression that can alter endothelial function or the release of mediators that influence the behaviour of endothelial and smooth muscle cells [16]. For example, increased shear stress is considered a major event in the pathogenesis of PHT and vascular remodelling in congenital left-to-right shunt. Similarly, creation of A-V access for HD therapy may increase both CO and pulmonary blood flow, alter shear stress and contribute indirectly to the development of PHT.

ET-1 is a potent vasoconstrictor and a powerful mitogen that has been related to PHT in many ways [8,9,17]. ET-1 levels are increased in humans with PHT [11]. High arterial levels compared with venous levels suggest that it is produced in the lungs [17]. Enhanced expression of the ET-1 gene and its receptor subtypes (ETA and ETB) has been demonstrated in lungs of...
humans with PHT [9,17]. Increased ET activity has also been reported in uraemia [8,9,17]. The current study does not indicate a role for ET-1 in the pathogenesis of this novel type of PHT. Nevertheless, taking into account that ET-1 is known to be secreted by the endothelial cells toward the vascular smooth muscle cells in a polar way, we do not exclude the possibility that local concentrations of ET-1 are enhanced in the pulmonary tissue of HD patients with PHT. This notion is supported by the findings that bosentan, an ET-1 antagonist, significantly reduced PHT in a 19-year-old woman with ESRD [18].

As we have suggested previously, an additional mechanism that may underlie the elevated PAP in HD patients may be haemodynamic changes associated with the placement of A-V fistula. The increase in CO due to enhanced venous return to the heart and subsequently exaggerated pulmonary blood flow following the creation of the A-V shunt indicate that these haemodynamic alterations may also play a crucial role in the development of PHT in these patients. This notion is supported by the findings that bosentan, an ET-1 antagonist, significantly reduced PHT in a 19-year-old woman with ESRD [18].

Fig. 3. Pulmonary arterial pressure (PAP) and cardiac output (CO) in HD patients with pulmonary hypertension (PHT) before and after kidney transplantation (A and B, n = 11), or A-V access compression (C and D, n = 8). *P < 0.05 compared with the relevant value before either transplantation or A-V closure.

As we have suggested previously, an additional mechanism that may underlie the elevated PAP in HD patients may be haemodynamic changes associated with the placement of A-V fistula. The increase in CO due to enhanced venous return to the heart and subsequently exaggerated pulmonary blood flow following the creation of the A-V shunt indicate that these haemodynamic alterations may also play a crucial role in the development of PHT in these patients. This notion is supported by our findings that PAP and CO decreased significantly after successful kidney transplantation, as well as after short A-V access compression. Since the PAP was reduced comparably in kidney recipients whether the fistula was opened or closed, despite a more remarkable decline in CO in the latter, these data suggest that non-haemodynamic factors may be involved in the beneficial effects of kidney transplantation. These factors may include improvement in their inflammatory status following the immunosuppressive therapy, cytokines, toxins, vascular remodelling, endothelial function and others. Therefore, in the present study, reduction in PAP following reduction in CO seems to be due not only to the flow dependency of PAP but also to a decrease in pulmonary resistance [19]. Clarkson et al. [20] recently reported PHT of 60 mmHg in an ESRD patient receiving chronic HD therapy via an aneurysmal brachicephalic A-V fistula. In line with our finding, surgical ligation of the A-V fistula and insertion of a tunneled semi-permanent internal jugular dialysis catheter reduced the PAP to 30 mmHg. We confirmed this observation, with significant reduction of PAP values (sometimes even to normal range) following temporary A-V compression by sphygmomanometer among eight HD patients, and noted normalization of PAP values following successful kidney transplantation in nine additional patients. From these data, we may conclude that the presence of both uraemia and A-V access-mediated elevated CO is mandatory for the development of PHT. The data presented by Clarkson et al. [20] indicate an unusual pattern of pulmonary hypertensive disease in HD patients, almost complete reversibility following CO reduction or amelioration of the uraemia. This reversibility of the PHT is surprising considering the data concerning the remodelling of pulmonary vessels in PHT. Although
pathological studies are absent, this reversibility of the PHT suggests that the pulmonary vasculature of the study population only underwent minor remodelling changes, if any.

Development of PHT in ESRD patients due to endothelial dysfunction and A-V access-mediated haemodynamic changes involves a therapeutic dilemma. PHT is associated with increased morbidity and mortality regardless of its aetiology. Similarly, HD patients with PHT had significantly higher mortality rates. Calcium channel blockers and angiotensin-converting enzyme inhibitors alone or in combination are likely to ameliorate endothelial dysfunction. On the other hand, poor response of PHT to medical therapy and reversibility of the PHT in this population of patients following A-V access closure or kidney transplantation are a call to refer affected patients to other modes of dialysis without A-V access, such as peritoneal dialysis, or to kidney transplantation.

In summary, the present study demonstrates that some uraemic patients on chronic HD therapy via A-V access exhibit decreased NO production. This endothelial dysfunction reduces the capacity of the pulmonary circulation to maintain the A-V access-mediated elevated CO and contribute to the development of PHT.

Conflict of interest statement. None declared.

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