Uraemic pruritus and exposure to di(2-ethylhexyl)phthalate (DEHP) in haemodialysis patients

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Abstract Uraemic pruritus is a frequent and disabling symptom in patients on dialysis. The pathogenesis of uraemic pruritus is nevertheless still obscure. We investigated whether di(2-ethylhexyl)phthalate (DEHP), the most commonly used plasticizer in polyvinylchloride (PVC) haemodialysis tubings, is a possible pathogenetic factor in uraemic pruritus. Serum concentrations of DEHP and its major derivatives mono-(2-ethylhexyl)phthalate (MEHP), 2-ethylhexanol (2-EH) and phthalic acid (PA) were determined in uraemic patients before and after a haemodialysis session and compared with the occurrence and intensity of pruritus in these patients. Twenty-one patients on regular haemodialysis for at least 6 months were examined. The severity of uraemic pruritus was assessed using a standard questionnaire (pruritus score). The quantitative analysis of DEHP and its derivatives was carried out by GC/selected ion monitoring mass spectrometry. Fourteen out of 21 patients (66%) complained about uraemic pruritus to a variable degree. The post-dialysis serum concentrations of DEHP, MEHP and 2-EH were significantly higher than the corresponding pre-dialysis values, whereas the post-dialysis concentrations of PA (0.122 ± 0.078 μg/ml) were significantly lower than pre-dialysis levels (0.194 ± 0.101 μg/ml, P = 0.00068). Neither pre- nor post-dialysis serum concentrations of DEHP, MEHP, PA or 2-EH were correlated with the severity of uraemic pruritus. Additionally, serum concentrations of DEHP and its metabolites did not differ significantly in patients with and without pruritus. These findings suggest that patients on haemodialysis are regularly exposed to considerable amounts of DEHP and metabolites. Phthalic acid, one of the presumed end products of DEHP metabolism, might be eliminated at least in part by haemodialysis. The exposition to DEHP and metabolites during haemodialysis, as assessed by measuring serum concentrations, bears no immediate relation to the occurrence or intensity of uraemic pruritus.

Key words: uraemia; pruritus; haemodialysis; plasticizer; DEHP; phthalates

Introduction

Uraemic pruritus is one of the most frequent and disabling symptoms in chronic renal failure, afflicting up to 85% of patients on dialysis [1]. Despite two decades of clinical investigation into uraemic pruritus little is known about the underlying pathogenetic mechanisms, and effective therapeutic strategies are still elusive. It was speculated that pruritogenic compounds not cleared by dialysis might be responsible for uraemic pruritus. This seemed to be a plausible hypothesis, since the administration of activated charcoal, presumably absorbing organic compounds in the intestinal fluid, alleviated uraemic pruritus, at least to a certain degree [2,3]. The observation that the frequency and intensity of uraemic pruritus increase after the start of dialysis treatment raises the question of whether the administration of pruritogenic substances or the dialysis-induced release of endogenous pruritogenic compounds is responsible for the development of uraemic pruritus. The release of endogenous substances, such as histamine, was thoroughly investigated in uraemic pruritus, with conflicting results [4–6]. In contrast, few data exist concerning exposure to xenobiotics and uraemic pruritus. One of the most important compounds known to be administered in continuous ambulatory peritoneal dialysis (CAPD) [7,8] and haemodialysis (HD) [9,10] is di(2-ethylhexyl)phthalate (DEHP), a plasticizer for polyvinylchloride (PVC), which is added to improve the flexibility of the material. Many medical tubings and storage bags, such as blood lines in haemodialysis and bags for CAPD fluid, are made of PVC. In these devices, the plasticizer may amount to up to 40% [11]. Since DEHP is not
chemically bound to the polymer, it leaches out of the plastic matrix and enters the systemic circulation of patients undergoing HD and CAPD. One of the major metabolites of DEHP, phthalic acid (PA), is known for its irritative effects on the skin [12].

The purpose of this study was to evaluate the relationship between the degree of exposure to DEHP and its main metabolites (mono-(2-ethylhexyl)phthalate (MEHP), 2-ethylhexanol (2-EH) and phthalic acid (PA)) and the occurrence and intensity of pruritus in haemodialysis patients.

**Subjects and methods**

**Patients**

Twenty-seven adult patients on regular HD treatment were evaluated for suitability to the study. Patients with a pre-existing skin disease (n = 3)—except usual cutaneous findings in uraemia, such as xerosis or ecchymosis—or patients unable to complete the questionnaire (n = 2) or taking antihistaminic medication (n = 1) were excluded from the study. The remaining 21 patients who were included had various renal diseases and had been undergoing haemodialysis (three times a week for about 4 h) for at least 6 months. They were treated using a Fresenius dialyser (polysulfone F6, F8 or HF60) and Fresenius blood lines. All patients gave written consent to participate in the study.

**Methods**

The patients were investigated after a long interval, i.e. 3 days, after the last dialysis. Blood samples were taken before and after a 4-h dialysis session. For the determination of the pre-dialysis concentrations of DEHP, MEHP, PA and 2-EH, 10 ml of blood was taken during the puncture of the arteriovenous shunt or the Goretex using a polyethylene-plastic syringe. After 4 h of dialysis, a second sample of 10 ml of blood was taken from the arterial port. All samples were immediately transferred to glass tubes, centrifuged, and the serum specimens were stored at —20°C until analysis. Additional blood samples were taken before dialysis to measure the serum concentrations of triglycerides and cholesterol in the course of routine laboratory investigation.

**Analytical methods**

Following the addition of the internal standards (di(n-octyl)phthalate (DOP), 0.5 μg; mono(n-octyl)phthalate (MOP), 0.5 μg; and n-octanol, 0.25 μg), aliquots of the serum samples (0.5 ml) were acidified to pH 2–3 and extracted twice with ethyl acetate (1.5 ml). The extracts were dried, concentrated under nitrogen and treated successively with diazomethane and BTSFA (N,O-bis(trimethylsilyl) trifluoroacetamide) to convert MEHP and MOP into the corresponding methyl esters and 2-ethylhexanol and n-octanol into the trimethylsilyl ethers, respectively.

Quantitative analysis of DEHP and its hydrolysis products was performed by selected ion monitoring gas chromatography/mass spectrometry, operating the mass spectrometer (Finnigan 4000 with Incos data system, coupled to a Carlo Erba gas chromatograph) in a combined positive and negative ion chemical ionization mode. Following on column injection and separation of the compounds on a 30-m capillary column (J&W Scientific), the [M-CH3]⁺ fragments of the TMS derivatives of 2-ethylhexanol and n-octanol were recorded in the positive mode. After the alcohols were eluted from the column, the mass spectrometer was switched to the negative mode to monitor the negative ions at m/z 148, which are characteristic for phthalate derivatives. Filament emission current and electron energy were maintained at 220 μA and 100 eV, respectively. The ion source temperature was 250°C, and the gas chromatograph interface was held at 280°C. Methane was used as reactant gas (0.04 kPa). The column oven temperature was programmed as follows: 40°C (1 min), 20°C min to 80°C, and 10°C min to 320°C (5 min). Helium was used as carrier gas (80 kPa).

The limit of quantification was approximately 1 ng/ml serum for DEHP, MEHP and PA, and 10 ng/ml serum for 2-ethylhexanol.

**Pruritus assessment**

All patients were questioned concerning pruritus at the time of the investigation. If this symptom was present, details regarding its intensity, its temporal relationship to dialysis, and its location (e.g. extremities, back or head) were noted together with the statements made in the following questionnaire to obtain a pruritus score.

**Pruritus score.** For the evaluation of pruritus, a modified score of Liu Jing Duo [1987] was employed. The intensity and distribution of uraemic pruritus and the frequency of sleep disturbances caused by pruritus were monitored as follows.

1. **Intensity:** itching without the need to scratch for its relief was scored as 1 point, with the need to scratch but without excoriations as 2 points, unrelieved by scratching or accompanied by excoriations as 3 points. Pruritus causing total restlessness received 5 points.

2. **Distribution:** itching at less than three locations was scored as 1 point, more than two locations as 2 points and generalized itching as 3 points.

3. **Sleep disturbance:** each episode of waking up due to itch was scored as 2 points (maximum 10 points).

The scores for intensity and distribution of pruritus were recorded and multiplied separately for the morning and the afternoon, so that a maximum of 15 points could be achieved for both time periods. The final score (40 points maximum) was obtained by adding the score of sleep disturbance and the intensity–distribution product.

**Statistical analysis**

For statistical analysis, the Sofistat® statistical program was used. In addition to the Wilcoxon matched pairs test for the comparison of the serum concentrations of DEHP, MEHP, PA and 2-EH before and after dialysis, the Kruskal–Wallis test was applied for the comparison of serum values of non-paired biochemical parameters. The Spearman rank correlation test was used to test the relationship between the itch score and the serum concentrations of all biochemical parameters measured. The data were expressed as mean±SD. P values less than 0.05 were considered to be significant.

**Results**

**Pruritus**

Fourteen out of 21 patients (66%) complained about pruritus at the time of the interview. The pruritus was
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most severe during or shortly after dialysis in eight patients and unrelated to dialysis in six. The pruritus was most severe in six out of 14 patients in the legs or arms, in six patients on the back and in two patients on the face.

**Phthalic esters**

The concentrations of DEHP and its major metabolites MEHP, PA and 2-EH in the serum of patients and controls are shown in Fig. 1. The serum concentrations of DEHP, MEHP and 2-EH at the end of the dialysis session were significantly higher than the corresponding levels at the beginning. The serum concentrations of PA, however, dropped significantly during dialysis (from 0.194 ± 0.101 to 0.122 ± 0.078 µg/ml, \( P = 0.00068 \)). No correlation was found between serum triglyceride or cholesterol levels, age or duration of dialysis and the pre- or post-dialysis concentrations of DEHP, MEHP, PA or 2-EH (data not shown).

The serum concentrations of DEHP, MEHP, PA and 2-EH of patients with and without pruritus did not differ significantly (Table 1). No correlation was found between the concentrations of DEHP, MEHP, PA or 2-EH and the intensity of pruritus as assessed by the score (Figs. 2 and 3).

**Discussion**

Phthalate esters, such as DEHP, are the most commonly used plasticizers in a wide variety of medical and technical products and have been detected in water, soil and food. As a consequence they are incorporated in the human body and can be detected in the blood [13]. DEHP and its hydrolysis products are rapidly eliminated by the kidney and in the faeces in healthy subjects [13]. Patients with impaired renal function and patients requiring dialysis treatment have an increased body burden of plasticizers, presumably for two reasons: firstly, as a result of the exposure to plastic materials during dialysis, and secondly, due to a decreased urinary elimination of these compounds.

![Fig. 1. Concentrations of DEHP, MEHP, PA and 2-EH in the serum of 21 patients before and after a 4-h HD session (mean ± SD). * \( P < 0.05 \).](https://academic.oup.com/ndt/article-abstract/11/12/2439/1867311)
It has been shown that the serum concentrations of DEHP and its hydrolysis products are considerably higher in HD patients than they are in CAPD patients [7,8]. When compared to data from Pollack et al. [9] and Gibson et al. [10], the serum concentrations of DEHP, MEHP and PA measured in the present study are of a similar magnitude. As the serum concentrations of DEHP, MEHP and 2-EH are rising during dialysis, it can be presumed that: (i) these compounds are taken up in the course of dialysis, and (ii) they are not sufficiently cleared during the session. These assumptions are supported, at least in part, by the data obtained by Pollack and coworkers [9]. Whether degradation of the DEHP administered during the dialysis treatment contributes significantly to the quantity of MEHP and 2-EH in the blood of the patients remains to be clarified. PA, a likely end product of DEHP metabolism, does however seem to be filtered effectively by dialysis, as indicated by the post-dialysis concentrations which are significantly lower than the corresponding pre-dialysis values. Therefore, dialysis seems to be an important route of elimination for this compound. Similar observations have been made in CAPD patients where dialysate concentrations of PA increased during dwell time [7].

The biological consequences of increased long-term exposure to phthalic esters are still unknown. Experimental data obtained in animals suggest that these compounds have teratogenic [14] and cystogenic effects [15], at least in rodents. Fraccasso and coworkers found that direct peritoneal contact with phthalic esters leads to ultrastructural changes of the peritoneum of rats and affects ultrafiltration [16]. Human data are sparse. Hilman and coworkers reported on polytransfused babies who had died of cardiac death and in whom very high concentration of DEHP were found in cardiac tissue [17]. Whether some symptoms occurring in dialysis patients are related to an increased exposure to plasticizers has not been intensively studied. No data are published regarding the role of xenobiotics, such as plasticizers, in the pathogenesis of uraemic pruritus.

About two-thirds of the haemodialysis patients participating in this study complained about pruritus of differing severity. This is in accordance with reports from others [18–20] and previously published data of our group [6]. Although not validated yet, the employed questionnaire seems to be—with some modifications—a widely used tool [21–23] appropriate for obtaining meaningful data on the occurrence and intensity of pruritus in dialysis patients.

The pathophysiology of uraemic pruritus has not yet been clarified. Four possible causes have been most thoroughly investigated: (i) stimulating factors, such as histamine [4–6,24], middle-sized molecules, as judged by dialysis efficacy [23,25], and PTH [26,27]; (ii) facilitatory factors, such as a scaly skin [28,29] and serological derangements [1,6,19,30]; (iii) neuropathic changes and receptor proliferations [31]; and (iv) disturbances in central modulation [32]. None of these possible causes has so far been unequivocally verified.

If a new pathogenetic model of uraemic pruritus is developed, it must explain the occurrence of uraemic pruritus in the early stages of renal failure, and the similar prevalence of uraemic pruritus in patients on HD and CAPD [6]. For elevated serum concentrations of plasticizers occur even in patients with renal insufficiency not requiring dialysis [9] and can be detected in the blood of patients on HD [8–11] and CAPD [7,8], plasticizers would meet the above mentioned criteria. Moreover, uraemic pruritus is often related to the haemodialysis procedure [6], which is, as previously shown, accompanied by a substantial exposure to DEHP [9].

Although phthalate esters such as DEHP are suggested to be of low acute toxicity [33], there are some reports on irritant and sensitizing properties of these substances [34,35]. PA, one of the major metabolites of DEHP, has been shown to be a potent skin irritant.
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[12]. Therefore the exposure to phthalate esters during dialysis would have theoretically qualified as a possible pathogenetic factor in uraemic pruritus.

Our study however fails to provide support for the hypothesis that high levels of DEHP or its main metabolites are responsible for uraemic pruritus. Most importantly, we found that the serum concentrations of DEHP and its metabolites are not significantly different in patients with and those without uraemic pruritus. Moreover, we did not find a correlation between pre- and post-dialysis concentrations of phthalate derivatives and the severity of pruritus, as assessed by the pruritus score. It should be kept in mind, however, that the serum concentration does not necessarily reflect the total body burden of a compound which may play a role in the development of uraemic pruritus. Therefore, additional studies must be carried out to investigate whether increased phthalate ester tissue concentrations are present in patients with uraemic pruritus.

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