Bis(2-ethylhexyl) Phthalate Concentrations in the Serum of Hemodialysis Patients

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Bis(2-ethylhexyl) phthalate is extracted from artificial kidneys, both in vivo and in vitro. Perfusion of whole blood through arterial-venous tubing (that supplied with the dialyzer) for 1 h in vitro yielded 3.23 mg of the compound, while similar perfusion of the tubing plus the artificial kidney yielded 6.10 mg. Its mean concentration after dialysis in patients undergoing hemodialysis was 751 µg/liter of serum. Patients who had undergone more than 50 hemodialysis treatments showed significantly higher postdialysis concentrations than patients who had undergone fewer. Uptake of bis(2-ethylhexyl) phthalate by the blood during a dialysis session followed two patterns. One set of patients showed a maximum concentration at 3 h, the second set showed a steady increase until the end of dialysis. Bis(2-ethylhexyl) phthalate appeared to be rapidly cleared from blood, most being removed within 5 to 7 h of completion of dialysis.

Additional Keyphrases: artificial kidney · toxicology · butyl benzyl phthalate · plasticizers · polyvinyl chloride tubing · dialysis dementia

A widely used plastic in the medical field is polyvinyl chloride. A rigid material, it can be made more flexible by adding plasticizers, more commonly bis(2-ethylhexyl) phthalate (DEHP). DEHP may constitute 20 to 40% by weight of a finished product.

DEHP has been shown to be leached from polyvinyl chloride into protein solutions or whole blood that comes into contact with the plastic. This migration has been demonstrated in vitro from blood transfusion bags (1) and hemodialysis tubing (2). It has been measured in blood of patients undergoing cardiac bypass (3, 4) and patients receiving platelet transfusions (5) as well as in the blood of patients undergoing hemodialysis treatments (6, 7).

The toxicity of phthalate esters has been reviewed (8, 9). In general, DEHP appears not to be very toxic. However, subtle effects on various enzyme systems may be important, especially in cases of chronic exposure (10–14).

We undertook to quantitate DEHP in serum during hemodialysis, to determine the rate at which it appeared in the blood as well as the rate of its disappearance from the blood after hemodialysis. Also, we determined the rates at which DEHP can be extracted by whole blood from the hemodialysis tubing and from the hemodialysis tubing and cartridge together, in vitro.

Materials and Methods

All glassware was prepared by soaking it in chromic acid for 4 h, rinsing with distilled water, and drying at 280 °C overnight.

Blood was sampled into glass syringes and transferred to glass tubes, which were then sealed with screw caps lined with aluminum foil. After centrifugation, the serum was removed and stored at −15 °C until analysis.

We developed the following procedure for measuring DEHP concentrations. For each sample, 2.0 ml of serum was extracted three times with 5.0-m1 portions of chloroform/methanol solution (2/1 by volume). The combined chloroform extracts were evaporated under nitrogen and the residue was dissolved in 5.0 ml of ethyl acetate, and then batch treated with 500 mg of alumina (Fisher Scientific Co., Fairlawn, N. J. 07410). The ethyl acetate was decanted and the alumina was rinsed with two additional 5.0-ml portions of ethyl acetate, which were added to the eluate, filtered through glass wool, and evaporated under nitrogen. This final residue was dissolved in 200 µl of hexane, which contained butyl benzyl phthalate as an internal standard. The internal standard was added at this step in the procedure, because butyl benzyl phthalate and DEHP have different extractabilities.

A 5.0-µl aliquot of this solution was analyzed by gas chromatography with a Model 5710A dual-column, flame ionization gas chromatograph (Hewlett-Packard, Inc., Avondale, Pa. 19311), with a 183 × 0.63 cm (2 mm i.d.) glass column containing 3% Dexsil 300 GC on 100/120 Supelcoport. Operating temperatures were: oven 200 °C, detector 300 °C, and injector 250 °C. Nitrogen (60 ml/min) was the carrier gas. For quantitation we used an Infotronics Model CRS-309 electronic in-
integrator (International Technical Instruments, Inc., Boulder, Colo. 80301). Peak identity was verified by combined gas chromatography/mass spectrometry with a Model 1015D quadrupole mass spectrometer (Finnigan Corp., Sunnyvale, Calif. 94086) and by proton magnetic resonance spectroscopy with a Model T-60 NMR instrument (Varian Associates, Inc., Palo Alto, Calif. 94303).

Some of the patients' samples were analyzed concurrently for cholesterol and triglycerides with a dual-channel continuous-flow system (AutoAnalyzer II; Technicon Instruments Corp., Tarrytown, N.Y. 10591). Cholesterol was determined on a zeolite–isopropanol extract by a modified Liebermann–Burchard procedure (15). Triglyceride was measured according to Kessler and Lederer (15) on the same extract.

Blood for two in vitro studies was collected from two men. The collection system was made entirely of glass, except for a small piece of polyvinyl chloride tubing (2 cm) used to connect the needle to the glass tube. The blood was heparinized and stored in glass bottles.

The Model EX-23 hemodialyzer (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa. 19406) was used in this study; the tubing used was that supplied with the dialyzer.

In the first in vitro study the dialyzer was bypassed, the arterial and venous tubings were connected together and perfused with 280 ml of blood for 1 h. The temperature was 22 °C, the flow rate 150 ml/min. A 10-ml sample was withdrawn from the blood reservoir before the start of perfusion, another just after the initial passage through the system, and others 5, 10, 15, 20, 30, 45, and 60 min later. Each time a sample was taken, 10 ml of blood was added to the reservoir, so that the total volume remained constant. In the second study, the perfusion was repeated with fresh arterial-venous tubing and with the EX-23 dialyzer connected in the circuit. The dialyzer was thermostated at 32 °C in a water bath, flow rate was 150 ml/min, and the blood volume was 420 ml. Sampling times and techniques were the same as in the previous perfusion.

For the in vivo studies, 10-ml blood samples were drawn by puncture of a cephalic vein of men and women volunteers. The clinical diagnoses of 27 of the patients were: chronic glomerulonephritis (n = 8), chronic pyelonephritis (n = 4), diabetic nephropathy (n = 4), hypertension plus nephrosclerosis (n = 3), lupus nephritis (n = 2), polycystic kidney disease (n = 2), postoperative acute tubular necrosis (n = 2), and rapidly progressive glomerulonephritis (n = 2). Fifteen samples were from patients who had had fewer than 50 dialysis treatments and 13 from those with more. Fifteen of the patients were men and 14 were women. Clinical diagnoses for two of the patients were unavailable, as was the total number of dialysis treatments for one patient.

For the measurement of DEHP concentrations relative to elapsed dialysis time, samples were drawn at various times during a dialysis session, usually at 1, 3,
and 5 h. The rate at which DEHP is removed from the blood was measured by sampling for as long as 4 h after the dialysis session.

For the arterial-venous comparison, samples were withdrawn from the dialysis tubing at the arterial side (as the blood left the patient) and immediately thereafter from the venous side (as the blood re-entered the patient). A dialysate consisting of a pooled sample from dialysis baths of several patients was extracted with the chloroform/methanol mixture, the solvent evaporated, and the residue reconstituted as were the serum samples.

Supplemented serum samples used in the recovery and precision studies were prepared by adding known amounts of DEHP in ethanol to a pooled sample of serum from normal volunteers. This mixture was then sonicated (Biosonik Ultrasonic Probe, Bronwill Scientific, Rochester, N. Y. 14603) at a sonic intensity of 60 for eight intervals of 30 s each, with 90 s between sonications.

The dialysis tubing, dialysis membrane, and its plastic mesh support were each checked for the presence of DEHP by removing a portion from an unused unit and extracting it with diethyl ether at room temperature for 15–20 min. The ether was then evaporated and the residue was reconstituted in hexane for gas-chromatographic analysis or in deuterochloroform for nuclear magnetic resonance spectroscopic analysis.

Results

The identity of the material being measured was determined by two independent methods. Methane chemical ionization mass spectra of the material appearing at the same retention time as DEHP in the gas-chromatographic analyses of post-dialysis samples from three different patients were superimposable on that of reagent-grade DEHP. Essentially no material in pre-dialysis samples from these same patients had the same retention time as DEHP. Typical gas-chromatographic analyses of a pre- and post-dialysis sample are shown in Figure 1. The nuclear magnetic resonance spectrum of the material isolated from the extraction of the dialysis tubing was also superimposable on the nuclear magnetic resonance spectrum of pure DEHP.

The precision of the gas-chromatographic analysis was estimated from the results of 10 successive 5.0-μl injections of butyl benzyl phthalate in hexane, 3.59 mg/liter. The CV was 3.0%. Analytical recovery of DEHP from the serum and the overall precision of the method were estimated by repeated analyses done on two different DEHP-supplemented serum samples. Five analyses of the first sample showed an average recovery of 69%, 792 μg/liter from 1126 μg/liter. The SD of the recovery was 60 μg/liter (CV, 7.5%). Eight analyses of the second sample showed an average recovery of 62%, 624 μg/liter from 1005 μg/liter. The SD was 32 μg/liter (CV, 5.2%). The overall recovery of the method is therefore in the range of 60–70%. The concentrations reported have not been corrected for this recovery.

In the in vitro studies of DEHP leaching from the dialysis tubing alone during perfusion and then of the dialysis tubing plus the EX-23 dialyzer, the total DEHP leached at each time was calculated by multiplying the DEHP concentration found by the total blood volume and then adding the DEHP removed in previous sampling. The results are shown in Figure 2. The total amount of DEHP was 3.23 mg from the perfusion of the tubing and 6.10 mg from the perfusion of the entire system.

Samples from various parts of the artificial kidney were tested for the presence of DEHP. A 220-mg section of the membrane was analyzed by gas chromatography and exhibited a peak with the retention time of DEHP (18–19 min). The amount leached was approximately 4.3 μg/g of membrane. A 390-mg section of the plastic mesh support used to separate the membrane layers was also analyzed. The amount of DEHP leached was approximately 2.5 μg/g of support. A large amount of DEHP was easily extracted from a 3-cm section of dialysis tubing.

The dialyzability of DEHP has not been demonstrated. Its molecular weight is 390, and so it would be expected to cross the dialysis membrane. However, the artificial kidney is submerged in a 100-liter bath, which makes the determination of dialyzed DEHP quite difficult. To find out whether or not DEHP is in fact dialyzable, we collected a small amount of ultrafiltrate from baths used with several patients. A large peak with the retention time of DEHP was seen on gas-chromatographic analysis of this material. Because the ultrafiltrate was a pooled sample and no data on patients were available, we made no further effort to quantitate the amount.

Analysis of the isotonic saline intravenous solutions
used to prime the artificial kidney showed no detectable DEHP (<20 µg/liter). Five control samples taken from normal subjects had no detectable DEHP (<50 µg/liter for serum samples).

The number of dialysis treatments that various patients had received ranged from as few as 1 to as many as 1300. Durations of dialysis treatments ranged from 3 to 8 h; most patients were dialyzed for 5 h per session. A total of 99 sera from these patients were analyzed. During the early phase of this study, samples were drawn immediately before the start of dialysis and just after completion of the dialysis session. After we determined that significant pre-dialysis concentrations are present only rarely, the pre-dialysis sample was discontinued.

Twenty-eight post-dialysis samples had a mean concentration of 751 ± 396 (SD) µg of DEHP per liter of serum, with a range of 250–1946. Serum samples from patients who had received fewer than 50 hemodialysis treatments showed a mean post-dialysis DEHP concentration of 558 ± 221 µg/liter (n = 15); samples from those who had undergone more than 50 treatments had a mean value of 973 ± 446 µg/liter (n = 13). These means differ significantly at the 95% confidence level by Student’s t test.

When 29 post-dialysis samples were compared according to the sex of the patient, the males showed an average DEHP concentration of 719 ± 361 (range) µg/liter (n = 15) and the females 767 ± 440 µg/liter (n = 14), a nonsignificant difference.

In an effort to determine the rate at which DEHP accumulates in the blood during dialysis, we sampled blood of six patients before, at 1 and 3 h during, and after dialysis, and plotted the change in DEHP concentration with time during dialysis (Figure 3). In three of the subjects the DEHP concentration was greatest at about 3 h and then decreased upon further dialysis, while in three other patients it increased throughout the dialysis. The averages of the concentrations were 475 µg/liter for 1 h, 806 for 3 h, and 923 for post-dialysis samples.

Once the mean post-dialysis concentrations had been determined, we measured the decrease in DEHP concentration after the end of the dialysis session (Figure 4). The mean values were 606 µg/liter immediately after dialysis, 323 at 30 min, 167 at 1 h, and 145 at 3 h after completion of dialysis. By extrapolation of the curves in Figure 4, it can be seen that most of the DEHP present in the serum at the completion of dialysis is likely to be gone in 5 to 7 h.

Marcel (16) has shown a positive correlation between triglyceride and DEHP concentrations in blood stored in plastic packs. On comparing the post-dialysis triglyceride and cholesterol concentrations in the serum of 21 patients with their DEHP concentrations, we found no correlation.

We corrected the DEHP concentrations found in samples taken at the end of dialysis sessions for the patients’ hematocrit but this yielded no improvement in the consistency of the data. Our results reported here do not include this correction.

To determine the amount of DEHP leached into circulating blood during an actual dialysis session, we measured DEHP concentrations in blood samples drawn just before the blood entered the patient (venous) and just after it left the patient (arterial). The difference
in these two values was considered to reflect the amount of DEHP leached from the dialysis system by the patient's blood in a single passage. Two patients, sampled after 2.75 h of dialysis, showed differences between arterial and venous concentrations of 264 and 541 μg/liter, respectively. If one assumes a mean flow rate of 200 ml/min, the amounts of DEHP leached during 1 h of dialysis were 3.16 and 6.5 mg.

Discussion

In our initial attempts at analyzing for DEHP we found large amounts present in procedure blanks and control samples. Once the very stringent clean-up procedure discussed in the Methods section was used, these contaminations were eliminated.

During a 1-h in vitro perfusion of arterial-venous tubing with whole blood, we found that 3.23 mg of DEHP was extracted into the blood; 6.10 mg was leached from the tubing plus the artificial kidney in the same interval. Since our analysis of an ultrafiltrate sample demonstrated that DEHP may be removed by dialysis, we expected that the concentration of DEHP would be lower when the artificial kidney was present. However, analysis of the membrane and plastic mesh supports of the artificial kidney showed that these materials may themselves contribute additional DEHP to the circulating blood.

The average serum DEHP concentration for patients after 1 h of dialysis was 475 μg/liter and post-dialysis sera reached a mean DEHP concentration of 751 μg/liter. If one only assumes a 25% hematocrit, a 5-liter total blood volume and no extravascular distribution, it can be estimated that almost 3 mg of DEHP is present in a patient's blood immediately after dialysis. This would correspond to at least 250-mg yearly dose in the case of a patient dialyzed twice a week. Because renal excretion has been shown to be a major pathway for excreting DEHP and its metabolites (17), much of this 250 mg, in addition to the DEHP absorbed during the dialysis, may accumulate in patients who lack kidney function.

The large range of serum DEHP values (250–1946 μg/liter) was not completely unexpected. Many factors may influence the actual concentrations of DEHP found in the serum: hematocrit, platelet counts, lipid concentrations, and the hepatic function. The importance of this last factor was seen in one of our patients whose post-dialysis concentration of DEHP in the serum exceeded 2000 μg/liter during the acute phase of hepatitis. Another possible source of variation may be the polyvinyl chloride itself. Even though the hemodialyzers used by the patients in this study were all of the same brand, they were from many different lots.

Post-dialysis samples from patients who had undergone more than 50 hemodialysis treatments were found to have an average of almost 50% more DEHP per liter of serum than those patients who had undergone fewer treatments. In patients undergoing long-term hemodialysis there are many physiological changes that may be responsible for increased post-dialysis DEHP concentrations, as compared to those on such treatment for a shorter time. Among these are impaired liver function, decreased hematocrit, and changes in platelet counts. Moreover, repeated exposure to DEHP might alter the body's ability to metabolize it. Long-term studies on the same patients may help to correlate DEHP concentrations with length of dialysis treatment.

Disappearance of DEHP from blood following a single intravenous dose has been reported to be biphasic by Schulz and Rubin (17), who suggested that the initial sharp decrease in concentration may be due to the rapid uptake of DEHP by a specific tissue in the body. In our case DEHP is being delivered to a patient for 5 h during a dialysis. Perhaps tissue uptake or metabolism, or both, occurs throughout the dialysis at a near constant rate. Our results show that once dialysis is completed, the net clearance of DEHP from the bloodstream decreases as less and less DEHP is available.

Correlation of DEHP concentrations with disease states evidently will require long-term studies. For example, serum DEHP concentrations apparently reflect liver dysfunction. Dialysis patients have frequent occurrences of hepatitis and we found that DEHP in serum increased dramatically in a patient during the acute phase of this disease. We have indirect evidence for a possible relationship between dialysis dementia and DEHP from a study on peritoneal dialysis patients that is now in progress: exposure of these patients to plastic is minimal, preliminary findings indicate no measurable DEHP concentrations (18), and dialysis dementia has not been reported for these patients.

Plastic tubing contains other additives besides plasticizers, such as anti-oxidants, ultraviolet stabilizers, and fire retardants. These compounds do not have as great a tendency to leach out as does DEHP; however, they are soluble in DEHP and so may accompany it. Animal toxicity studies on material leached from actual tubes may give a clearer picture of possible effects resulting from long-term contact of a patient with polyvinyl chloride tubing.

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References


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