Donor exposure to the plasticizer di(2-ethylhexyl)phthalate during plateletpheresis

Christoph Buchta, Claudia Bittner, Paul Höcker, Maria Macher, Rainer Schmid, Christoph Seger, and Markus Dettke

BACKGROUND: Di(2-ethylhexyl)phthalate (DEHP) is a plasticizer that is contained in most PVC devices, including apheresis disposables. Because DEHP can be extracted from apheresis disposables as the blood passes through the apheresis device, DEHP exposure was determined in healthy donors undergoing plateletpheresis performed with commercially available apheresis systems.

STUDY DESIGN AND METHODS: The study population consisted of 36 healthy PLT donors undergoing plateletpheresis with either continuous or discontinuous apheresis devices. Serum concentrations of DEHP were determined from peripheral blood obtained before and after plateletpheresis, with gas chromatography-mass spectroscopy.

RESULTS: Plateletpheresis performed with standard collection disposables resulted in a median increase of 232 percent of serum DEHP compared to levels before apheresis, corresponding to a total amount of DEHP exposed during a single apheresis of a median of 6.46 (range, 1.8-20.3) mg per kg of body weight. Endogenous levels of triglycerides showed a positive correlation with the amount of DEHP released. Increase in serum DEHP was short-term as serum DEHP rapidly returned to levels obtained before apheresis within 3 hours after completion of the apheresis course. Donor exposure to DEHP led to no variation in liver cell function within 48 hours after plateletpheresis.

CONCLUSION: Commercial plateletpheresis disposables release considerable amounts of DEHP during the apheresis procedure, but the total dose of DEHP retained by the donor is within the normal range of DEHP exposure of the general population.

ABBREVIATION: DEHP = di(2-ethylhexyl)phthalate.

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formed in many blood banks and transfusion services. During plateletpheresis, donor blood passes through the collection disposables of the apheresis device before the blood returns to the donor. Parts of these collection disposables are manufactured with PVC containing DEHP. During blood passage through the apheresis instrument, DEHP can be resolved in the blood, leading to leaching of DEHP into the donor. To assess DEHP exposure associated with PLT donation, in the present study we analyzed the serum levels of DEHP in donors undergoing routine plateletpheresis.

MATERIALS AND METHODS

Study population and plateletpheresis procedures
Thirty-six healthy PLT donors (23 men, 13 women) participated in the study after written informed consent was obtained. Seventeen donors underwent plateletpheresis with continuous (“double-needle”) apheresis machines; in 19 donors PLT collection was performed with discontinuous (“single-needle”) cell separators. Plateletpheresis was performed with different systems (in 5 subjects, the Spectra apheresis system [Haemonetics Corporation, Braintree, MA]; in 6 donors, the Com.Tec cell separator and the C5L PLT set [Fresenius HemoCare, Bad Homburg, Germany]; and in 6 donors, the Amicus cell separator with the R4R2314 Amicus double-needle apheresis kit [Baxter Healthcare Corporation, Deerfield, IL]). Discontinuous plateletpheresis was performed with the following system (MCS + LN 9000-220-E [Haemonetics Corporation, Braintree, MA], with the 994 CF-E extended storage PLT and apheresis set [Haemonetics UK Ltd., Bothwell, UK]). Detailed donor characteristics and apheresis related key data are summarized in Table 1.

Collection of blood samples
Peripheral blood was sampled before and after completion of plateletpheresis. In four donors, additional blood sampling was performed at 1, 3, 6, 24, and 48 hours after completion of the apheresis course. To avoid any secondary contamination with DEHP derived from syringes manufactured with PVC, blood was taken through metal canulas and collected in glass tubes. After collection, the blood was centrifuged and the serum was stored at –20°C.

Chemicals
DEHP was used as internal standard and was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Methanol and acetonitrile were purchased in from Merck (Darmstadt, Germany), n-heptane was obtained from Fluka (Buchs, Switzerland), and n-decane 99+ percent was purchased from Aldrich (Steinheim, Germany). Deionized water was prepared by a water purification system (Milli-Q, Millipore; Vienna, Austria).

Extraction procedure
For extraction of DEHP from serum samples, a method described elsewhere was modified. In brief, 500μL of serum was transferred to a 10-mL glass tube, and 2.1 mL of 5 percent aqueous methanol was added. Proteins were precipitated with 2.5 mL of acetonitrile, and 50 μL of internal standard was added before shaking the mixture on a vortex. DEHP and the internal standard were extracted into 2.5 mL of n-heptane by shaking for 10 minutes. After centrifugation at room temperature (1000 × g 10 min), the organic layer containing DEHP was removed. After addition of n-decane, the samples were evaporated to a final volume of 200μL. One microliter of the final extract was injected into the gas chromatography system.

DEHP analysis
Serum samples were analyzed with gas chromatography-mass spectrometry. Separation was achieved on a gas chromatograph (Fisons GC 8000, Fisons Instruments SpA, Milan, Italy), with a 5 percent diphenyl-95 percent dimethylpolysiloxane capillary column (25 m length, 0.2 mm inner diameter, 0.2μm film thickness; Optima 5MS, Macherey-Nagel, Düren, Germany). The helium flow rate was set at 3.0 mL per minute. The following operating settings were used: an injector temperature of 280°C and an oven temperature programmed from 160°C (held for 1 min) at 30°C per minute to 200°C and at 6°C per minute to 280°C (held for 10 min). DEHP was quantified with a mass spectrometer (QMD 1000, Carlo Erba, Milan, Italy). Mass spectrometry conditions were selective ion monitoring of the ions m/z 279, 167, and 149 for DEHP and m/z 328, 326, and 254 for the internal standard. The ion source was operated in the electron ionization mode (70 eV, 250°C). The limit of detection was 10 ng of DEHP per mL of serum.

<table>
<thead>
<tr>
<th>TABLE 1. Patient characteristics and apheresis-related key data</th>
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<tr>
<td><strong>Single-needle devices</strong></td>
</tr>
<tr>
<td>Number of donors (male/female)</td>
</tr>
<tr>
<td>Total blood volume (L)*</td>
</tr>
<tr>
<td>Total plasma volume (L)*</td>
</tr>
<tr>
<td>Inlet blood flow (mL/min)*</td>
</tr>
<tr>
<td>Total blood volume processed (L)*</td>
</tr>
<tr>
<td>Total apheresis time (min)*</td>
</tr>
</tbody>
</table>

* Data are expressed as median (range).
Estimation of total amount of DEHP exposed during plateletpheresis

Given a recovery of 100 percent in plasma, the individual amount of DEHP exposed during plateletpheresis was estimated according to the formula

\[ \text{Total amount of DEHP exposed} = (a - b) \times c, \]

where \( a \) is the serum level of DEHP as determined after apheresis, \( b \) is the serum level of DEHP as determined before apheresis, and \( c \) is the donor’s total plasma volume.

Statistical analysis

Data are expressed as median and range. Differences in serum DEHP as assessed before and after plateletpheresis were compared by t test for paired samples and by U test, when appropriate. A \( p \) value of less than 0.05 was considered significant.

RESULTS

Increase in serum DEHP after plateletpheresis

To assess the effect of plateletpheresis on serum DEHP levels, we compared DEHP levels obtained after completion of the apheresis procedure to those obtained before apheresis (Fig. 1). Plateletpheresis resulted in a marked increase in serum DEHP. Median serum levels of DEHP increased from baseline levels of 92.2 (range, 5.9-219.6) to 213.8 (range, 7.3-716.1) ng per mL, corresponding to a median DEHP exposure of 6.46 (range, 1.8-20.3) \( \mu \)g per kg of body weight. DEHP was independent of the apheresis technology used. In single-needle apheresis machines, the median DEHP exposure was 6.4 (range, 1.9-19.5) \( \mu \)g per kg of body weight compared to 7.2 (range, 2.0-20.3) \( \mu \)g per kg of body weight when continuous apheresis machines were used (\( p = 0.85 \)). There was no association between increase in serum DEHP and apheresis-related modifications of the apheresis procedure. Serum DEHP increased independently of the inlet blood flow (\( p = 0.82 \)), total amount of blood processed (\( p = 0.89 \)), or total apheresis time (\( p = 0.79 \)) (data not shown).

Follow-up kinetic analysis of serum DEHP revealed that the increase of DEHP was short-term. DEHP levels returned to values obtained before apheresis within 3 hours after plateletpheresis and remained at baseline levels within the following 48 hours after completion of the apheresis setting (Fig. 2).

Correlation between increase in serum DEHP and endogenous serum lipid concentration

To assess whether endogenous factors can influence DEHP release during plateletpheresis, we correlated the concentration of serum triglycerides and serum cholesterol to the amount of DEHP released. There was a weak association between the concentration of serum triglycerides and the relative increase in serum DEHP (Fig. 3; \( r^2 = 0.24, p = 0.03 \)). In contrast, no correlation existed between increase in DEHP and the concentration of serum cholesterol (\( r^2 = 0.0003, p = 0.8 \)) (data not shown).

No changes in liver function measures after plateletpheresis

To assess possible changes in liver enzymes after exposure to DEHP, we monitored the serum levels of the liver parameters glutamic oxaloacetate transaminase, glutamic pyruvic transaminase, gamma-glutamyltranspeptidase, and cholinesterase for up to 48 hours after completion of plateletpheresis. None of these measures showed any significant variations during the observation period (\( n = 8 \) donors; Table 2).

DISCUSSION

DEHP is a ubiquitous plasticizer that can leak from PVC-derived materials. Because disposables used for platelet-
pheresis are manufactured from PVC containing DEHP, the aim of the present study was to determine the exposure to DEHP in normal donors undergoing plateletpheresis. Plateletpheresis caused up to a 10-fold increase in serum DEHP compared to levels before apheresis. Given a 100 percent recovery of DEHP in serum, the median amount of DEHP exposed during a single plateletpheresis procedure was estimated to be 6.5 mg per kg of body weight, with a broad interindividual variation ranking from 1.8 to 20.3 mg per kg of body weight. Despite this significant exposure to DEHP, the total amount of DEHP leaching during plateletpheresis is at least 3 log lower than the estimated DEHP exposure of 14.8 mg per kg per day during hemapheresis previously calculated by Doull et al.11 One explanation for the discrepancy between hemodialysis and plateletpheresis is that in hemodialysis the total blood volume processed is between 20 and 30 L, which is 4 to 10 times higher compared to the 3 to 5 L of total blood volume processed during normal plateletpheresis.

We observed a positive association between the levels of serum triglycerides and the amount of DEHP retained by the patients is up to 3.1 mg per kg of body weight.15 One explanation for the discrepancy between hemodialysis and plateletpheresis is that in hemodialysis the total blood volume processed is between 20 and 30 L, which is 4 to 10 times higher compared to the 3 to 5 L of total blood volume processed during normal plateletpheresis.

TABLE 2. Variations in liver function measures

<table>
<thead>
<tr>
<th></th>
<th>GOT† (U/L)</th>
<th>GPT (U/L)</th>
<th>γ-GT (U/L)</th>
<th>Cholinesterase (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline*</td>
<td>12 (9-18)</td>
<td>13 (10-35)</td>
<td>23 (8-42)</td>
<td>6.4 (3.5-7.8)</td>
</tr>
<tr>
<td>After plateletpheresis</td>
<td>10 (9-15)</td>
<td>11 (7-33)</td>
<td>20 (8-30)</td>
<td>5.6 (2.9-7.4)</td>
</tr>
<tr>
<td>24 hr after plateletpheresis</td>
<td>10 (9-18)</td>
<td>13 (9-37)</td>
<td>22 (7-39)</td>
<td>6.2 (3.2-8.6)</td>
</tr>
<tr>
<td>48 hr after plateletpheresis</td>
<td>11 (10-16)</td>
<td>13 (11-28)</td>
<td>22 (8-39)</td>
<td>5.5 (3.3-8.5)</td>
</tr>
</tbody>
</table>

* Data are expressed as median (range).
† GOT = glutamic oxaloacetate transaminase; GPT = glutamic pyruvic transaminase; γ-GT = gamma-glutamyltranspeptidase.

example, during hemodialysis, the total dose of DEHP retained by the patients is up to 3.1 mg per kg of body weight.15 One explanation for the discrepancy between hemodialysis and plateletpheresis is that in hemodialysis the total blood volume processed is between 20 and 30 L, which is 4 to 10 times higher compared to the 3 to 5 L of total blood volume processed during normal plateletpheresis.

We observed a positive association between the levels of serum triglycerides and the amount of DEHP retained by the donors. This can be explained by the chemical properties of the plasticizer. As a lipophilic substance, leaching of DEHP is enhanced at higher plasma lipid levels, as already demonstrated in other clinical settings.4,21 Variations in the apheresis course, such as modifications in inlet blood flow or the total blood volume processed, showed no significant influence on the
DEHP exposure during plateletpheresis

The amount of DEHP released. These data are not in contrast to previous findings demonstrating a time and temperature dependency on the extraction rate of DEHP. Plateletpheresis was performed at ambient room temperature, and the total apheresis time varied only marginally between the different apheresis settings (Table 1). The influence of physical factors on the DEHP extraction rate might therefore be too small to be detected in our setting. The small variations in total apheresis time may also explain the lack of differences in the amount of DEHP released from tubing sets of the various apheresis systems tested, despite the increased contact surface area between blood and tubing sets in continuous apheresis machines compared to discontinuous cell separators.

Kinetic analysis revealed that the increase in serum DEHP was only short-term. DEHP levels returned to values obtained before apheresis within 3 hours after completion of plateletpheresis. DEHP is converted by plasma and liver enzymatic actions to mono(2-ethylhexyl)phthalate, and this metabolite is more toxic than DEHP. In an animal model, the T50 of DEHP has been reported to be 30 minutes. The observed rapid disappearance of DEHP from circulation is therefore in agreement with the quick metabolism of DEHP, although it should be concerned that serum levels of mono(2-ethylhexyl)phthalate were not determined in the present study. An alternative explanation may involve a multicompartment model where DEHP is accumulated in various tissues, including the spleen, the liver, the lungs, and abdominal fat.

Toxicity associated with acute administration of DEHP appears not to be very important. In accordance with these findings, we did not observe any short-term effects of DEHP exposure on liver cell function after plateletpheresis. However, animal studies on the chronic exposure to high doses of DEHP suggest a broad range of effects on several organ systems. This includes adverse effects in the reproductive tract, the kidneys, the lungs, and the heart. Furthermore, DEHP can induce changes in hepatic morphology and biochemical functions, and chronic DEHP exposure is associated with an increased incidence of liver tumors in DEHP-treated animals. Although these data implicate potential hazards of prolonged exposure to high doses of DEHP, it should be a concern that DEHP is a ubiquitous environmental contaminant. In the general population, the total amount of DEHP exposure is estimated to be in the range of 3 to 30 μg per kg of body weight and per day. Based on these data and in view of our results assuming a median DEHP exposure of 6.5 μg per kg of body weight during plateletpheresis, the additional DEHP uptake can be calculated to be in the range of 5 to 20 percent above the daily exposure. In some donors, the additional exposure might even exceed 50 percent of the normal daily ingested and inhaled uptake. However, this estimation has some limitations. Our calculation is based on the assumption of a single-compartment model with a 100 percent recovery of DEHP in serum and does not include the possibility of an accumulation of DEHP in tissue. Furthermore, this estimation does not include any DEHP metabolism during the time of the apheresis donation. However, despite these uncertainties, it should be concerning that the FDA has set the tolerable intake value of DEHP at 600 mg per kg of body weight and per day. This value is approximately 30 times higher than the highest serum levels of DEHP determined in the present study. Our data therefore gave no evidence for a critical DEHP exposure during plateletpheresis. However, for safety issues, longitudinal studies must exclude any potential hazard of repeat DEHP exposure in repeat plateletpheresis donors.

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REFERENCES


