Increased Di-2-Ethyl Hexyl Phthalate (DEHP) During Extra Corporeal Circulation Using Different Oxygenators

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Abstract

The amount of Di-2-Ethyl Hexyl Phthalate (DEHP) released from the components of Extra Corporeal Circulation (ECC) systems is studied in vitro, ex vivo and in vivo experiments.

The in vitro and ex vivo studies showed that the ester is released from the Polyvinyl Chloride (PVC) used. For the in vivo study, 55 patients were divided into two groups. Both groups each used a different oxygenator (either a TMO or a BOS 10). Immediately after surgery a significant increase in DEHP was observed. After a 24 hours period (without ECC) the amount decreased.

A significant difference in the total phthalate ester content between the two groups revealed that the TMO patients had a higher DEHP blood level concentration than those using the BOS 10 oxygenator.

Introduction

A major portion of the plastic used in medical devices consists of polyvinyl chloride (PVC). Its flexibility comes from the addition of a plasticizer: di-2-ethyl hexyl phthalate (DEHP). This phthalate ester (about 40% of the total PVC content) is not chemically bound to the plastic but is physically linked: DEHP can thus be leached from the plastic when placed in contact with certain substances. In this manner DEHP can be found in total blood[1] and in red blood cells[2] stored in plastic bags. We have found the presence of DEHP on G-immunoglobulins from patients having undergone several plasmaphereses.[3]

Extensive studies have been carried out on DEHP with regard to its acute toxicity in a variety of experimental animals. The acute oral LD_{50} in the rat is 31 gr/kg.[4] Information pertaining to the subacute and chronic toxicity of DEHP has been derived largely from the earlier reports by Schaffer et al.[5] These early investigations revealed a "no-effect" oral dose of approximatively 60 mg/kg/day in the rat.

The DEHP was implicated as causing non viral hepatitis in dialysis patients.[6,7] Other reports have indicated that skin eruptions resembling porphyria cutanea may be induced by the use of PVC tubing.[8,9,10]

The presence of DEHP in the blood of hemodialyzed patients,[11] and the importance of PVC in medical-tubing and in the extracorporeal blood circulation system for use in open heart surgery has brought us to investigate the concentration levels of DEHP in extracorporeal devices and in patients who were submitted to such extracorporeal circulation (ECC). In order to estimate the release of DEHP, several ECC devices were tested.

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Materials and Methods

This study is divided in two parts: one is in vitro and ex vivo, and the other is in vivo. We have chosen three types of ECC systems which we use regularly in open heart surgery: the Bentley system which uses a BOS 10 oxygenator[a] and the Travenol systems with TMO oxygenator, and Variflo 6LF bubble oxygenator[b].

1. *In vitro studies.* All components are preassembled and delivered sterile, ready for use by the manufacturers and custom packs are identical in quality and quantity of tubing except for the added accessories necessary for their use (flexible plastic bags for use with membrane oxygenators). Each custom pack was divided into comparable components and was tested as a closed circuit, either with heptane which is a powerful DEHP extractor or with blood, imitating clinical conditions.

The concentrations of DEHP in the oxygenator were compared to those found in the meter lengths of plastic-tubing and accessories.

A SARNS[c] roller pump was used to test each component with occlusion performed according to the manufacturer's instructions, in a closed circuit for 4 hours.

When using silicone tubes no phthalate ester was released. The silicone tubes were used with glass recipients in the closed circuit testing. Samples were taken every 15 minutes and were assayed for DEHP.

2. *In vivo studies.* In this study, ECC was used on 55 patients. The samples were assayed for the presence of the ester 5 minutes before the start of the ECC, 5 minutes after, and 24 hours after ECC. Blood samples were taken from a radial artery catheter.

The patients under study were divided into two groups. Group I consisted of those undergoing ECC with the TMO oxygenator and Group II included those using BOS 10.

The groups are comparable since:

—the circuits were identical, except for the oxygenators and accessories inherent with the system (flexible plastic bags, shunt);

—the types of interventions (Table 1), the mean duration of ECC, the average diuresis and transfusions from plastic bags which could interfere by contamination and falsify results are similar in both groups, as shown in Table 2.

TABLE 1
Type Of Interventions In Each Group

Type of interventions	With Travenol circuit	With Bentley circuit
Coronaries surgery	15	15
Valves surgery	9	12
Congenital diseases	1	3
Total	25	30

The priming volume for both groups was identical in quantity and quality and were always stored in glass prior to use.

3. *Estimation of DEHP.* In all cases DEHP was assayed with the same procedure: after liquid extraction with heptane, DEHP is estimated directly by gas chromatography (GLC)[12] with SE 30 columns on chromosorb WHP (80/100 mesh). Experimental conditions on the 2 meters column were as follows: oven temperature was set at 220°C, injection temperature at 250°C and detector at 260°C. The carrier gas was nitrogen with a flow rate of 30 ml/min. For each assay digermanium was used as internal standard, which has a molecular weight greater than DEHP with a retention time not interfering with that of DEHP.

The same extraction procedure was used either with patients or with in vitro experiments. The ester is extracted from serum by a chloroform/methanol mixture (20:1 v/v) (20 volumes of solvent for each volume of serum). After a complete evaporation to dryness under nitrogen, the residue was dissolved in a mixture of chloroform/methanol (1:1 v/v).

The DEHP ester was then separated from the solvent system by thin layer chromatography using fluorescent plates (Merck). The solvent system was composed of hexane, diethyl ether and acetic acid (90:10:1 v/v). DEHP was then dissolved in heptane and assayed by GLC.

Results

1. *Oxygenators.* Tested separately for a duration of 4 hours show that the BOS 10 tested in

[a] Bentley Laboratories, Inc., Irvine, CA 92714
[b] Travenol Laboratories, Deerfield, IL 60015
[c] SARNS, Inc., Ann Arbor, MI 48103

TABLE 2
Mean Value Compared In Both Groups (± Standard Deviation): Time Of ECC, Diuresis, Bags Number Of Blood And Globules Perfused During The ECC

Devices	ECC time min	Diuresis ml/h	Blood bags number of unit	Red blood cells bags number of unit
Group I (25 patients) TMO	84.5 ± 16.9	379.15 ± 65.67	1.37 ± 0.14	3.38 ± 0.28
Group II (30 patients) BOS 10	95.5 ± 17.44	405.45 ± 86.26	0.83 ± 0.17	2.93 ± 0.22

vitro with heptane does not release any phthalate ester, whereas the TMO tested ex vivo (because membranes are chemically attacked by heptane in vitro) releases a small quantity of DEHP (20 μmol/l), which most probably originate from different plastic cements used. The Travenol Variflo 6LF tested in vitro releases a great deal of DEHP (68000 μmol/l).

2. *Plastic tubing*. We tested the silicone tubing for traces of DEHP and found none. This allowed us to use this tubing for connections assuring no contamination by phthalates. The in vitro results are given in Figure 1, and show that the DEHP content of the Travenol (curve a) and Bentley (curve b) tubes are in fact practically the same.

However, in the Travenol circuit there are two flexible PVC bags which also release some DEHP (curve c) and this circuit requires a longer PVC tubing length. These two factors release a large quantity of DEHP (curve d).

3. *In vivo results*. In both groups there is a marked increase of DEHP near the end of ECC. This is shown in Table 3. The in vivo results confirm the in vitro ones, since the significant increase of DEHP in the Travenol circuit is 1‰ using Student's t-test, compared to only 5% with the Bentley circuit.

Discussion

In all cases we have seen that in ex vitro and in vitro studies the phthalate ester appearing in blood originated from the PVC and was extracted by the blood in contact with it within the first few minutes of contact. This quantity also increases proportionally as a function of time contact. The consequences are the following: on the one hand the quantity of DEHP appearing in blood can be toxic,[13,14] on the other hand the extraction of the ester from PVC tubing modifies its physical and chemical structure and tends to harden it. This loss of flexibility and compliance may lead to either breakage of tubing within the pump head or

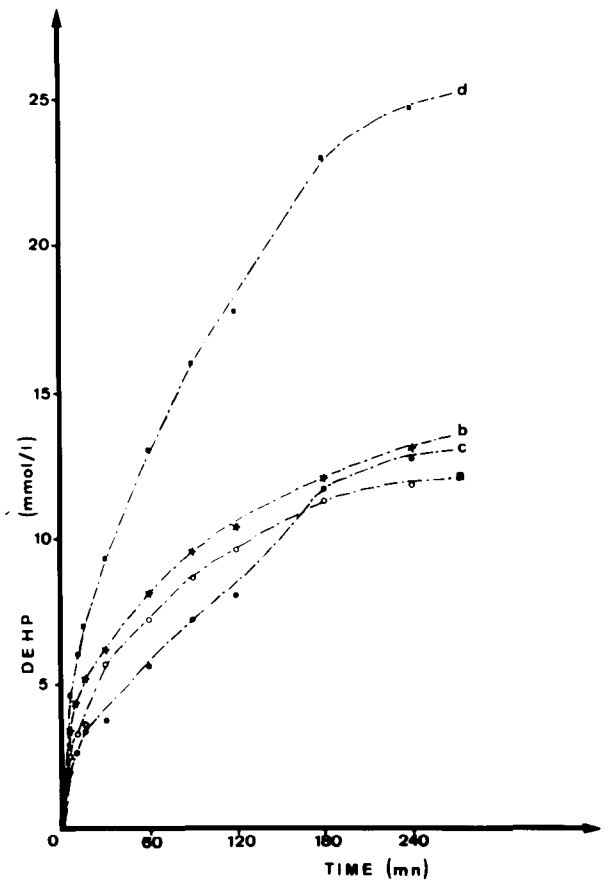

FIGURE 1. Concentration of DEHP released during 4 hours by the following systems. a) Travenol tubes (1.20 m) b) Bentley tubes (1.20 m) c) Travenol bags alone d) Travenol bags and Travenol tubes (d = a + c)

TABLE 3
DEHP Released During ECC

	Travenol circuit TMO		Bentley circuit BOS 10	
	DEHP concentrations μmol/l			
Patient data	5 mn before ECC	5 mn after ECC	5 mn before ECC	5 mn after ECC
1	28	29	7	21
2	21	35	25	42
3	18	40	30	43
4	26	24	48	63
5	17	43	58	72
6	29	33	7	10
7	20	49	22	37
8	18	37	6	12
9	39	45	25	48
10	25	76	6	12
11	40	61	11	11
12	31	35	25	37
13	32	104	80	160
14	27	19	55	92
15	11	19	23	38
16	15	45	15	16
17	55	42	24	13
18	18	28	17	16
19	17	26	11	18
20	20	38	17	20
21	10	17	9	15
22	13	31	8	6
23	17	28	19	9
24	42	52	49	12
25	80	94	29	26
26			40	48
27			7	8
28			10	5
29			7	9
30			9	6
Mean value ± Standard deviation	26.76 ± 3.09	42 ± 4.34	23.3 ± 3.39	30.83 ± 5.98
Student's t test	1‰		5%	

to unsuspected changes in stroke volume of the pump.

In both groups of patients, important quantities of DEHP are found in blood after ECC. The total concentration of this ester tends to decrease, and normalization is obtained 24 hours after the end of ECC.

The reason that DEHP is found in greater concentration in group I than in group II is that TMO system requires in addition to the standard tubing, flexible PVC bags needing supplementary lengths of PVC tubing.

Conclusion

Elimination of PVC tubing from ECC systems would seem to be of primary importance, since this plastic is the one responsible for the release of the phthalic acid ester in blood. Replacing it with silicone tubing which produces no DEHP would be convenient. An inconvenience is that a silicone circuit is a little bit more expensive. Advantages are, for one, the disappearance of phthalate esters in patients undergoing ECC, and the other is the reduction of the numbers of con-

nectors between silicone and PVC tubing which consequently reduces the risks of thrombosis and hemolysis.

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