Cyclohexanone contamination from extracorporeal circuits impairs cardiovascular function

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Extracorporeal circulation (EC; cardiopulmonary bypass, extracorporeal membrane oxygenation [ECMO], hemodialysis, hemofiltration) has become a primary tool in hospitals, providing critical life support in the face of cardiopulmonary or renal failure. However, EC also introduces a host of unique morbidities characterized by edema formation, cardiac insufficiency, autonomic dysfunc-

tion, and altered vasomotor function. We tested the hypothesis that cyclohexanone (CHX), a solvent used in production of extracorporeal circuits and intravenous (IV) bags, leaches into the contained fluids and can replicate these clinical morbidities. Crystalloid fluid samples from circuits and IV bags were analyzed by gas chromatography-mass spectrometry to provide a range of clinical CHX exposure levels, revealing CHX contamination of sampled fluids (9.63–3,694 μg/l). In vivo rat studies were conducted (n = 49) to investigate the effects of a bolus IV infusion of CHX vs. saline alone on cardiovascular function, baroreflex responsiveness, and edema formation. Cardiovascular function was evaluated by cardiac output, heart rate, stroke volume, vascular resistance, arterial pressure, and ventricular contractility. Baroreflex function was assessed by mean femoral arterial pressure responses to bilateral carotid occlusion. Edema formation was assessed by the ratio of wet to dry organ weights for lungs, liver, kidneys, and skin. CHX infusion led to systemic hypotension; pulmonary hypertension; depressed contractility, heart rate, stroke volume, and cardiac output; and elevated vascular resistance (P < 0.05). Mean arterial pressure responsiveness to carotid occlusion was dampened after CHX infusion (from +17.25 ± 1.8 to +5.61 ± 3.2 mmHg; P < 0.05). CHX infusion led to significantly higher wet-to-dry weight ratios vs. saline only (3.8 ± 0.06 vs. 3.5 ± 0.05; P < 0.05). CHX can reproduce clinical cardiovascular, neurological, and edema morbidities associated with extracorporeal circulatory treatment.

ventricular contractility; autonomic nervous system; vasoconstriction; edema

Cyclohexanone [CHX; C6H10(==O)] is an organic solvent used in the production of polyvinyl chloride (PVC) medical devices, including intravenous (IV) fluid bags and EC circuits. CHX easily migrates from PVC tubing and connections into fluids that come in contact with PVC (10, 12, 35, 37), irrespective of the type of IV fluid. CHX can leach from PVC bags and IV tubing into IV fluids in sufficient concentrations for CHX metabolites to be detected in neonatal urine samples (26). Therefore, it is likely that EC patients and patients receiving large fluid volume:patient volume quantities of IV crystalloid are at risk for significant CHX exposure, given that their full blood volumes circulate continuously through CHX-treated PVC tubing and are frequently augmented (+25% of original volume) with CHX-contaminated fluids.

The toxicity of PVC and CHX has been documented in animal models, leading to decreased cardiac and neural cell viability (9, 16), depressed cardiac contractility (16, 25), neurological abnormalities (23), edema (16), and death or moribund state (16, 23). However, these and other studies employed near-lethal CHX doses, and the routes of CHX administration also varied widely from study to study, making direct comparison to clinical IV CHX exposure virtually impossible. Thus, although there is mounting evidence that CHX is toxic to the cardiovascular system, there is scant data available regarding either the minimum CHX dose required to induce these effects or the mechanism(s) through which CHX may exert them.

To explore the possibility that CHX may serve as a mediator of the profound cardiovascular dysfunction seen in EC patients, it is critical to establish whether CHX—in clinically relevant doses—can reproduce these morbidities. Accordingly, the purposes of this series of experiments were to establish current values for CHX contamination in crystalloid fluid from IV bags and EC circuits and to test the hypothesis that a clinically relevant dose of CHX can reproduce the cardiovascular and neurological dysfunction and edema formation that commonly accompany EC support/treatment.

METHODS AND MATERIALS

Gas Chromatography-Mass Spectrometry Analysis

to quantify the degree of CHX contamination of fluids at the Johns Hopkins Medical Institutions, crystalloid samples from commercially available IV saline bags, lactated Ringer solution bags, cardiopulmonary bypass circuits, ECMO circuits, and hemodialysis circuits were collected and analyzed with gas chromatography-mass spectrometry (GC-MS). Briefly, 4 ml of each crystalloid sample was withdrawn, and deuterated CHX (dCHX) was added in (final concentration: 100 μg/l) as internal standard. The sample was extracted into 1 ml of methylene chloride, withdrawn, and analyzed with an HP5000 series GC-MS equipped with a Restek column (Rtx-1ms, 30 m × 0.25-mm inner diameter × 0.25 μm film thickness). Peak areas corresponding to mass-charge ratio (m/z) 98...
(CHX) and m/z 102 (dCHX) were integrated. A standard curve was prepared by adding CHX in concentrations ranging from 0 to 2,000 μg/l with 100 μg/l dCHX into an initial sample. Ratios of the two areas were used to calculate CHX concentrations.

In Vivo Anesthetized Rat Experiments

After experimental procedures were approved by the Animal Care and Use Committee at the Johns Hopkins Medical Institutions, 49 adult (3–6 mo) male Sprague-Dawley rats were obtained from Harlan Industries (Indianapolis, IN) and housed in a virus-free facility, receiving food and water ad libitum for a minimum of 3 days before experimentation. All rats were anesthetized intraperitoneally with either ketamine + acepromazine (120 mg/kg and 2.4 mg/kg, respectively) or thiopental sodium (100 mg/kg) before the start of the experiment.

In all experiments, rats received a bolus IV infusion of either saline (vehicle) or 21.6 μM CHX, given at 2.5% blood volume (assuming total blood volume of 80 ml/kg). This IV dose of CHX was selected as a conservative approximation of EC patient CHX exposure, based on the results of the GC-MS analysis (see Table 1). With cardiopulmonary bypass as the basis for this dosing model, initial bypass patient blood volume (5 l) is abruptly augmented by ~25% (1.25 l) with circuit fluids containing at least 210 μg/l CHX (or 2.16 μM). Thus, immediately after the patient goes on bypass, blood volume is 6.25 liters and contains a total of 262.5 μg of CHX. This results in an initial predicted blood CHX concentration of 42 μg/l. In transitioning these mathematical assumptions to an animal model, we modified this protocol to avoid the confounding effects of high volume load, thereby allowing us to fully unmask the effects of CHX on cardiovascular function. Thus we reduced the infusion volume by a factor of 10 (i.e., to 2.5% blood volume) and increased the CHX concentration by a factor of 10 (i.e., to 2.10 mg/l or 21.6 μM). This alteration allowed us to deliver a CHX load proportionately comparable to that seen in bypass but with only minimal volume load, as seen in the following calculation. Initial blood volume in a 500-g rat (assuming 80 ml/kg) is 40 ml. Therefore, a 2.5% blood volume equivalent of 21.6 μM CHX is equal to 1 ml of a 2.10 mg/l solution. After IV infusion of this CHX solution, the total blood volume is 41 ml and contains a total of 2.1 μg of CHX. This results in a predicted rat blood concentration of 51 μg/l, comparable to the 42 μg/l human value. This series of calculations supports three major assumptions of our study: 1) that our dosing procedure approximated a predicted adult EC patient CHX insult, 2) that our dosing procedure was a conservative estimate of EC patient exposure, because 210 μg/l was the lowest CHX concentration we detected in EC circuit fluids, and 3) that our dosing procedure (i.e., employing a bolus infusion of CHX instead of a steady drip) was a valid approximation of the abrupt exposure to CHX that bypass, ECMO, and dialysis patients undergo when they first are connected to the circuit tubing. After the experiments, rats were euthanized by CO2 asphyxiation.

Protocol 1: measurement of cardiovascular function. For measurements of resting cardiovascular function in anesthetized rats (CHX rats n = 8, saline rats n = 6), experiments were performed as previously described (27, 42) and measured cardiac output, heart rate, stroke volume, systemic and pulmonary vascular resistances, mean aortic pressure, mean pulmonary artery pressure, and three indexes of ventricular contractility [ejection fraction, change in ventricular pressure divided by change in time, normalized for instantaneous pressure (dp/dt-IP), and end-systolic pressure-volume relationship (ESPVR)].

Table 1. Cyclohexanone contamination in crystalloid from IV bags and EC circuits

<table>
<thead>
<tr>
<th>Source of Crystalloid</th>
<th>n</th>
<th>CHX, μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (0.9%) saline, 1 liter</td>
<td>9</td>
<td>164.8 (24.2–1,082)</td>
</tr>
<tr>
<td>Lactated Ringer solution, 1 liter</td>
<td>4</td>
<td>34.6 (9.63–68.1)</td>
</tr>
<tr>
<td>CPB</td>
<td>2</td>
<td>454 (210–698)</td>
</tr>
<tr>
<td>ECMO</td>
<td>2</td>
<td>2,016 (337–3,694)</td>
</tr>
<tr>
<td>Hemodialysis/filtration</td>
<td>1</td>
<td>2,100</td>
</tr>
</tbody>
</table>

Cyclohexanone (CHX) values are means (range) for n samples. IV, intravenous; EC, extracorporeal circulation; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation.

RESULTS

GC-MS Analysis Of CHX Contamination of Crystalloid Fluids

Sampled crystalloid fluids included normal saline (0.9%) and lactated Ringer solution from IV bags (n = 9 and n = 4, respectively), cardiopulmonary bypass circuit perfusate (n = 2), ECMO circuit priming fluid (n = 2), and continual renal replacement therapy (CRRT) circuit perfusate, used for either hemodialysis...
ysis or hemofiltration \((n = 1)\) (Table 1). IV bag contamination with CHX ranged from 24.2 to 1,082 \(\mu\)g/l in saline bags and from 9.63 to 68.1 \(\mu\)g/l in lactated Ringer solution bags. CHX contamination ranged from 210 to 698 \(\mu\)g/l in cardiopulmonary bypass priming fluid and from 337 to 3,694 \(\mu\)g/l in ECMO priming fluid and was 2,100 \(\mu\)g/l in renal dialysis priming fluid. On the basis of these values, 210 \(\mu\)g/l CHX was chosen as the standard for all subsequent in vivo rat model experiments.

**In Vivo Protocol 1: Effects of CHX on Resting Cardiovascular Function**

Before infusion of either saline or CHX, all baseline values were similar between saline and CHX groups \((P > 0.05)\). In the 60 min following saline infusion, none of the variables significantly changed from baseline; however, CHX infusion induced significant adverse changes in all cardiovascular variables (Figs. 1 and 2). Both stroke volume (Fig. 1A) and heart rate (Fig. 1B) values were significantly depressed after 1 h of CHX vs. saline alone (stroke volume, CHX vs. saline: 157 \(\pm\) 4 vs. 200 \(\pm\) 3 \(\mu\)l/beat, \(P < 0.001\); heart rate, CHX vs. saline: 287 \(\pm\) 11 vs. 358 \(\pm\) 4 beats/min, \(P < 0.001\)). These reductions resulted in 34\% decline in cardiac output in CHX rats over the course of the hour, significantly lower than in saline rats (CHX vs. saline: 44.8 \(\pm\) 1.7 vs. 71.6 \(\pm\) 1.5 ml/min, \(P < 0.001\); Fig. 1C). CHX infusion also caused an increase in systemic vascular resistance compared with saline values (CHX vs. saline:

![Fig. 1. Cardiovascular function at baseline and 60 min after intravenous infusion of saline or 21.6 \(\mu\)M cyclohexanone (CHX). SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; PAP, mean pulmonary artery pressure. A–E: saline \(n = 6\), CHX \(n = 8\). F and G: saline \(n = 3\), CHX \(n = 5\). *\(P < 0.05\), **\(P < 0.001\) vs. saline.](https://www.ajpheart.org/doi/10.220.33.3)
1.38 ± 0.05 vs. 1.02 ± 0.03 mmHg·ml⁻¹·min, P < 0.001; Fig. 1D). However, this increase in systemic vascular response was not sufficient to counter the drop in cardiac output, and mean aortic pressure exhibited a net hypertensive response to CHX vs. saline (CHX vs. saline: 61.6 ± 3.3 vs. 72.6 ± 1.3 mmHg, P < 0.05; Fig. 1E). Pulmonary vascular resistance also increased during CHX infusion vs. saline (CHX vs. saline: 0.44 ± 0.03 vs. 0.17 ± 0.01 mmHg·ml⁻¹·min, P < 0.001; Fig. 1F). This increase was more than sufficient to offset the decrease in cardiac output, and mean pulmonary artery pressure exhibited a hypertensive response to CHX vs. saline (CHX vs. saline: 18.8 ± 0.7 vs. 12.3 ± 0.2 mmHg, P < 0.001; Fig. 1G).

All indexes of ventricular contractility were also depressed 60 min after CHX vs. saline alone. LV and RV ejection fractions (an index dependent on both preload and afterload) were both significantly decreased after CHX vs. saline alone [LV, CHX vs. saline: 47.9 ± 2.5% vs. 68.1 ± 0.7%, P < 0.001; RV, CHX vs. saline: 52.4 ± 1.2 vs. 67.7 ± 0.4%, P < 0.001 (data not shown)]. LV and RV dP/dt-IP values (an index dependent on preload but independent of afterload) were also significantly decreased after CHX vs. saline alone [LV, CHX vs. saline: 54.8 ± 1.6 vs. 71.6 ± 0.8 s⁻¹, P < 0.001; RV, CHX vs. saline: 36.9 ± 1.8 vs. 68.6 ± 1.4 s⁻¹, P < 0.001 (data not shown)]. Finally, LV and RV ESPVR (an index independent of both preload and afterload) were significantly depressed after CHX vs. saline alone (LV, CHX vs. saline: 0.78 ± 0.01 vs. 1.67 ± 0.03, P < 0.001; RV, CHX vs. saline: 1.49 ± 0.12 vs. 3.60 ± 0.06, P < 0.001; Fig. 2).

**In Vivo Protocol 2: Effects of CHX on Cardiovascular Autonomic Function**

Before any infusions, saline and CHX groups exhibited comparable baroreflex pressor responses to bilateral carotid occlusion (Fig. 3), with significant differences between preocluded and occluded mean arterial pressures during baseline in both groups (saline: 113 ± 3.7 vs. 132 ± 5.6 mmHg, P < 0.001; CHX: 115 ± 7.0 vs. 132 ± 8.6 mmHg, P < 0.001). After both first and second saline infusions, the pressor response in the control group did not differ significantly from baseline (P > 0.05; Fig. 3A). However, after the first CHX infusion, the pressor response, although still significant (107 ± 7.3 vs. 119 ± 8.7 mmHg, P < 0.05; Fig. 3B), was less pronounced compared with baseline values (baseline occluded vs. 1st infusion occluded: 132 ± 8.6 vs. 119 ± 8.7 mmHg, P < 0.05; Fig. 3B). After the second CHX infusion, the pressor response was abolished (83 ± 10 vs. 88 ± 11 mmHg, P > 0.05; Fig. 3B) and significant hypotension had developed (P < 0.001).

**In Vivo Protocol 3: Effects of CHX on Edema Formation**

The wet-to-dry ratio of organ weights served as an index of tissue fluid retention, and thus edema formation. The ratios for all individual organs within each treatment group were pooled to create an overall index of systemic edema formation; the pooled ratio for the CHX group was significantly higher vs. the saline group (CHX vs. saline: 3.8 ± 0.06 vs. 3.5 ± 0.05, P < 0.001; Fig. 4). The breakdown of ratios by organ within each treatment group is illustrated in Fig. 4, inset; although the wet-to-dry weight ratios varied significantly among organ groups regardless of treatment, CHX treatment consistently resulted in higher edema formation within each organ group.

**DISCUSSION**

Data from the present study 1) provide a current estimate of CHX contamination in commercially available IV bags and EC circuits and 2) clearly indicate that the effects of 210 μg/l IV CHX (a concentration that conservatively approximates clinical exposure for EC patients) mirror the cardiovascular morbidities associated with EC: bradycardia/dysrhythmia (1, 4, 5, 19, 33, 38), depressed ventricular contractility (1, 2, 4, 5, 8, 11, 18, 19, 24, 33, 38, 39, 41), low cardiac output (7, 11, 14, 28, 31), high systemic and pulmonary vascular resistance (7, 13, 18, 29–31), hypotension/labile blood pressure (2, 7, 21), pulmonary hypertension (7, 29–31), impaired neurological (baroreflex) function (3, 5, 34, 38, 39), and edema formation (20, 32, 39).

The striking similarities of the present data with clinical cardiovascular morbidities associated with EC support/treatment suggest that CHX, as a contaminant leaching from IV bags and EC circuit tubing into EC circuit perfusate, may serve as a direct trigger for these morbidities in EC populations. Previous studies have pioneered the analysis of CHX contamination of saline, lactated Ringer solution, and dextrose solutions in PVC IV bags (10, 12, 35, 37); however, this study is...
the first to extend this leaching analysis to EC circuits and to use those circuit values to create an in vivo animal model evaluating the isolated cardiovascular effects of a clinically relevant EC CHX exposure. Given the variable degrees of contamination in different IV bags and EC circuits (9.63–3,694 µg/l), a conservative, yet clinically relevant, exposure level was chosen for the animal model: 210 µg/l. It is important to note that the CHX values tended to increase as the volume of the EC circuit decreased. Cardiopulmonary bypass values were lower than both ECMO and dialysis circuit values, because although all three circuits contained a similar number of CHX-welded joints, the volume of circuit fluid into which CHX leached was much higher in the bypass circuits, leading to a more dilute final concentration vs. either ECMO or dialysis values.

The cardiovascular insufficiency elicited by CHX was unmistakable in the 34% decrease in cardiac output. Although vascular factors can adversely affect cardiac output (e.g., decreased preload, increased afterload), the decrease in cardiac output in the present study was mainly attributed to a combination of cardiac factors: bradycardia and ventricular contractile dysfunction, leading to lower stroke volume (Fig. 1, A and B). In clinical situations, depressed ventricular contractility is almost universally managed with inotropes to prevent circulatory failure, and thus the extent of unmitigated contractile damage associated with EC treatment is not assessed. However, in the present study, the intent was to unmask the full effects of CHX without the confounding influence of pharmacological intervention, which revealed a significant drop in LV and RV contractility (see Fig. 2).

Ejection fraction is a commonly used, noninvasive clinical indicator of contractility; however, ejection fraction is also highly dependent on preload and afterload. A second index of contractility used in the present study, dP/dt-IP, is more widely used with in vitro research and is less dependent on afterload (although still confounded by preload). A third index of contractility, ESPVR (Fig. 2), is the most invasive but also the most transparent of the three indexes, completely independent of preload and afterload (36). Thus a wide spectrum of accepted contractility indexes were calculated in this study, all yielding the same conclusion—that CHX elicited significant ventricular contractile dysfunction, similar to that associated with EC treatment. The independence of ESPVR from preload and afterload was particularly critical in establishing the inherent cardiac nature of the problem; because the changes in arterial pressure and vascular resistance were so pronounced (see Fig. 1), it was essential to use a contractility index that would not be perturbed by other factors.

On the basis of the data collected in this study, it was not possible to discern whether the decreases in right and left heart contractility were due to a primary effect of CHX on cardiac myocytes or were secondary to a CHX-induced decrease in coronary flow. However, two recent studies (11, 24) suggest that the short-term ventricular dysfunction associated with hemodialysis is driven largely by a decrease in regional myocardial blood flow, and that the loss of ventricular function is proportional to the degree of hypoperfusion. Thus it is plausible that the increase in vascular resistance observed in the present study in response to CHX (see Fig. 1, D and F) may have resulted in coronary vasoconstriction sufficient to depress contractile function. Further testing of CHX-mediated changes in coronary flow is certainly warranted.

The observed increase in systemic and pulmonary vascular resistances suggests that significant vasoconstriction occurred.
throughout the body in response to CHX infusion. The moderate increase in systemic vascular resistance was offset by a proportionately greater decrease in cardiac output, resulting in systemic arterial hypotension. The far greater increase in pulmonary vascular resistance, on the other hand, more than offset the decrease in cardiac output and led to pulmonary hypertension, similar to that observed during and after EC treatment. A plausible explanation for the increased vascular resistance would be a loss of vasodilator function. Two studies by Nyhan and colleagues (29, 30) indicate that cardiopulmonary bypass elicits an increase in pulmonary vascular resistance and attendant pulmonary hypertension by abolishing endothelium-dependent vasodilation. Thus CHX may mimic the high vascular resistance/pulmonary hypertension associated with bypass through similar vasodilator mechanisms, although this theory requires further testing.

Three pieces of data from the present study suggest that CHX may also induce neurological dysfunction. 1) In protocol 1, heart rate decreased by 59 beats/min (Fig. 1B) from pre-CHX baseline without an overt bradycardic stimulus, suggesting vagal-sympathetic dysregulation of heart rate after CHX treatment. 2) In protocol 1, the decrease in mean arterial pressure after CHX infusion (Fig. 1E) should have acted as a moderate endogenous stimulus of the baroreflex and signaled a reflex tachycardic response in an effort to restore blood pressure. Instead, CHX infusion led to a decoupling of the heart rate-blood pressure relationship that normally typifies baroreflex control of mean arterial pressure. Thus CHX mimics the high vascular resistance/pulmonary hypertension associated with bypass through similar vasodilator mechanisms, although this theory requires further testing.

The final cardiovascular morbidity documented in the present study common to both CHX infusion and EC support/treatment was edema formation. In protocol 3, the wet-to-dry weight ratios from livers, lungs, kidneys, and skin were pooled to create an overall index of systemic edema formation in saline- and CHX-treated groups. The pooled ratio in the CHX group was 0.3 greater than the saline group (3.8 vs. 3.5), indicating a significant 9% increase in the wet-to-dry ratio after CHX infusion (Fig. 4). This increase represents a 9% wet weight gain, a value that is comparable to a 90-kg bypass patient gaining 8 kg of fluid while in the ICU—a plausible clinical scenario. Thus CHX infusion induces a reasonable degree of edema formation, comparable with what is observed in cardiopulmonary bypass patients. However, it was not possible, based on the data collected in this study, to distinguish whether Starling forces or endothelial barrier dysfunction (or both) were responsible for the movement of fluid from the vascular to extravascular space. Clarification of these mechanisms will require further study.

It is possible that CHX may contribute to the systemic inflammatory response syndrome (SIRS), a more global complication of EC support/treatment that encompasses not only severe cardiovascular insufficiency and edema but also complement activation, renal/liver failure, and pulmonary dysfunction. While there are several potential triggers of SIRS, many of them are unavoidable—intrinsic to the extracorporeal nature of the treatment. However, CHX is unique in that it would be an external risk factor for SIRS that could potentially be ameliorated. Further work is warranted to explore the full scope of systemic CHX toxicity and to determine whether CHX is, indeed, a novel trigger for SIRS.

The primary limitation of this study is that it was performed in an animal model while projecting its findings onto a human clinical population. However, the nature of the protocols in this study precluded their implementation in a clinical population because of ethical concerns. An in vivo rat model is a well-accepted experimental approximation for the human cardiovascular system, and the data obtained from this model provided valuable information regarding CHX, a potential source of significant clinical morbidity that is found in most commercially manufactured IV bags and EC circuits.

**Conclusions**

CHX toxicity has long been recognized in animal models but has never been considered capable of mediating a significant clinical response. Our data clearly correlate clinically relevant CHX exposure with the development of key cardiovascular morbidities associated with EC treatment, supporting the hypothesis that CHX is a toxic mediator that may play a role in the onset of EC-associated morbidities and contribute to the attendant deterioration. This study provides the foundation for further research into mechanisms driving this CHX-induced cardiovascular insufficiency, as well as the potential for CHX to serve as a trigger of SIRS, a trigger that may ultimately be easily remedied.

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The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

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