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Heating Injection Drug Preparation Equipment Used for Opioid Injection May Reduce HIV Transmission Associated With Sharing Equipment

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Background: London, Canada, experienced an HIV outbreak among persons who inject drugs despite widespread distribution of harm reduction equipment. Hydromorphone controlled-release (HMC) is the local opioid of choice. Injection drug preparation equipment (IDPE; ie, cookers and filters) is often shared and reused because of the perception that there is residual HMC in the IDPE after use. The purpose of this study was to investigate the mechanisms of HIV transmission in this context.

Methods: Residual hydromorphone, (controlled-release or immediate-release), remaining in the IDPE, was measured with liquid chromatography–tandem mass spectrometry, in conditions replicating persons who inject drug use. HIV was added to IDPE in the presence HMC, hydromorphone immediate-release, or microcrystalline cellulose (an HMC drug excipient). HIV viral persistence was measured by reverse transcriptase activity and infectivity of indicator Tzm-bl cells.

Results: Forty-five percent of HMC remained in the IDPE after the first aspiration of solution, with no change after heating. HIV persistence and infectivity were preserved in the presence of HMC, and less so with microcrystalline cellulose. Heating the IDPE rapidly inactivated HIV.

Conclusions: Sharing of IDPE is a potential means of HIV transmission. HMC encourages IDPE sharing because of the residual drug in the IDPE, and the HMC excipients preserve HIV viability. Heating IDPE before aspiration of the opioid may be a harm reduction strategy.

Key Words: HIV transmission, injection drug use, prescription opioid injection

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BACKGROUND

Injection of prescription opioids has reached epidemic levels in North America, resulting in an almost fourfold rise in prescription opioid-related substance use disorder treatment admissions in the United States between 2004 and 2014.[1,2] This epidemic has also been associated with several large outbreaks of HIV infection among persons who inject prescription opioids (PWID),[3–5] including one in London, Ontario, Canada, that resulted in the declaration of a public health emergency on June 14, 2016 (Fig. 1).[6] The control of these outbreaks generally depends on a multipronged approach of distribution of sterile injection equipment, opioid substitution therapy, and provision of antiretroviral therapy.[7] The outbreak in London was unusual because it occurred despite the city already having at baseline the highest distribution of sterile needles and syringes per capita in Canada and an opioid substitution therapy prescription rate in the top quartile for Ontario.[8] And, London has a government-funded, multidisciplinary HIV care program with access to funded HIV antiretroviral therapy. The reason for this outbreak despite the excellent pre-existing control programs was the subject of the current investigation.

A study of injection drug users in London identified hydromorphone controlled-release (HMC) capsules [marketed in over 15 countries on 5 continents with several brand names, including Hydromorph Contin or Palladone SR (Purdue Pharma, Stamford, CT) and Jurnista (Janssen-Cilag

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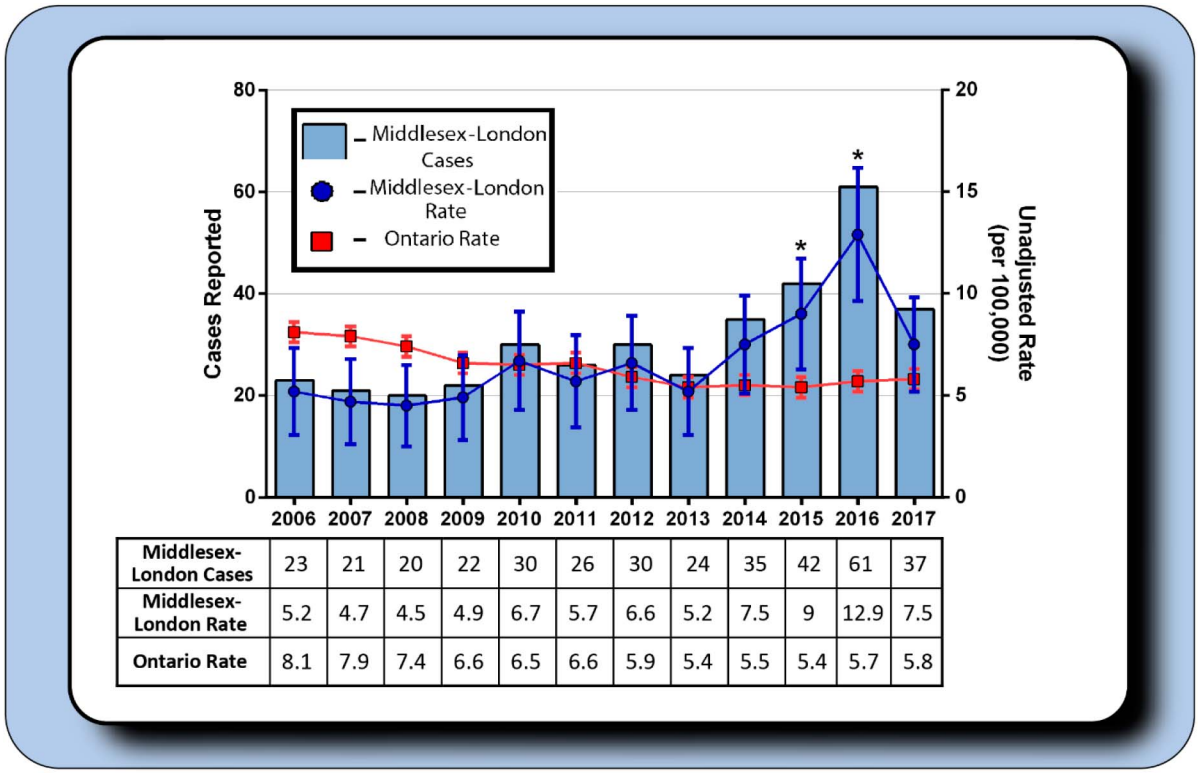

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Middlesex-London Cases	23	21	20	22	30	26	30	24	35	42	61	37
Middlesex-London Rate	5.2	4.7	4.5	4.9	6.7	5.7	6.6	5.2	7.5	9	12.9	7.5
Ontario Rate	8.1	7.9	7.4	6.6	6.5	6.6	5.9	5.4	5.5	5.4	5.7	5.8

FIGURE 1. Count and incidence of HIV infection in Middlesex-London and Ontario, 2006–2017. The Ontario and Middlesex-London HIV rates (per 100,000) are shown in the red and blue lines, respectively. Middlesex-London incident HIV cases per year are shown in the blue bars. *Denotes significantly higher ($P < 0.05$) incidence of HIV in Middlesex-London relative to Ontario.

Pty Ltd, Macquarie Park, New South Wales, Australia)] as the preferred opioid for illicit use.[9] Most PWID (79%) reported HMC use in the past 6 months.[9] Moreover, prescription rates of HMC capsules were unusually high in London compared with other regions in Ontario, which may indicate widespread availability of the drug for diversion and injection.[8]

HMC capsules have a low solubility in water, and thus, preparation of the drug for injection requires numerous steps (Fig. 2A).[10] The timed-released beads must be removed from the capsule and crushed, mixed with water in a cooker to create a slurry, and then drawn into a syringe through a cotton filter to remove visible particulate matter.[10] The filters, cookers, and water are referred to as the injection drug preparation equipment (IDPE). The high cost and large dose of hydromorphone in the controlled-release formulation, and the need to dissolve the capsule in a large volume of water, increase the likelihood that the IDPE will be shared between people.[10] The large volume of the solution can require multiple injections, and therefore, PWID are tempted to divide the injections among several people, while sharing costs. In addition, PWID report large amounts of nonsolubilized drugs are retained in the filter and cooker after the first use, thereby encouraging the reuse and sharing of used IDPE.[10,11]

Reuse of IDPE is achieved by adding water to the previously used cooker containing residual drug, placing a needle into the previously used filter, and aspirating the residual drug (the "wash"). Multiple PWID may participate in "serial washes," whereby the same filter/cooker/drug complex and needle/syringe are reused each time, with some PWID performing and sharing as many as 7 washes from a single preparation of HMC.[10,11] While PWID reuse and share the same IDPE, they often reuse their own, but do not share needles/syringes (Fig. 2).

Sharing of needles/syringes among PWID has been well established as a means of HIV transmission,[12] and so distribution of sterile needles/syringes has been a major focus of HIV prevention programs. However, sharing of IDPE is more common than sharing of needles/syringes.[13–17] In some centers, sharing of IDPE has been identified as the primary risk factor for hepatitis C virus transmission,[13,18–20] but its association with HIV transmission has not been widely studied. The incentives for sharing IDPE despite the existence of free IDPE distribution programs have also not been extensively studied. We recently demonstrated in a case–control study that sharing IDPE was independently associated with HIV infection and that negative beliefs about heating the IDPE were also associated with HIV infection.[21]

We propose that in a PWID population where IDPE is frequently shared, IDPE may become contaminated with HIV when an HIV-positive PWID reuses their own needle to perform another wash (including using the used needle to stir the solution and placing the needle into the filter to aspirate the fluid) (Fig. 2B).[22,23] HIV could be transmitted when the contaminated IDPE is shared with a second, HIV-negative PWID placing their own needle into the used filter/cooker to aspirate another wash. We further proposed that drug

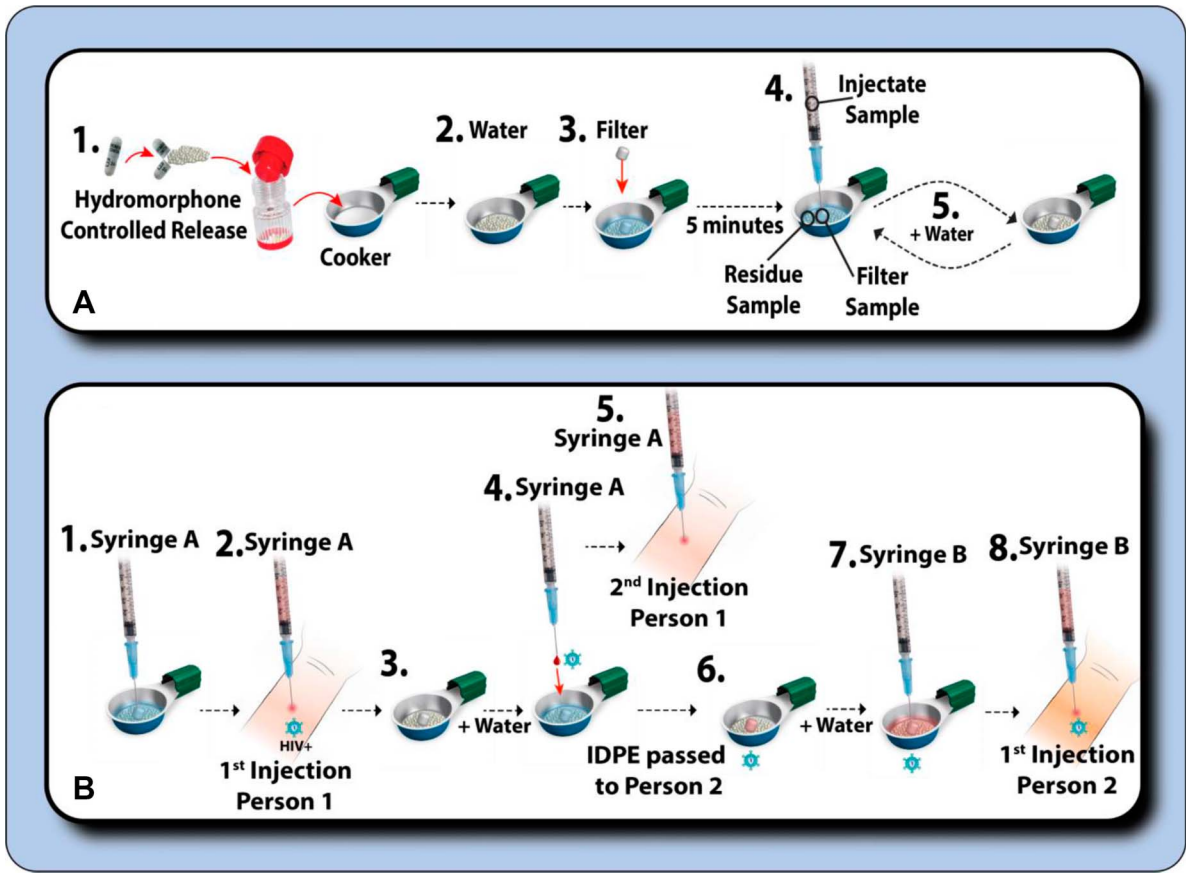

FIGURE 2. Method for preparing hydromorphone controlled-release for injection. A, 1. Open hydromorphone controlled-release capsule and empty time release beads into a pill crusher to crush the beads. Empty the crushed beads into a cooker. 2. Add approximately 1.5 mL of water to the cooker and mix using needle to stir. Allow the cooker to sit for 5 minutes at room temperature to further dissolve the opioid. 3. Place a cotton filter into the solution. 4. Place needle in the filter and draw up the solution into a syringe. Individuals would then inject this solution (injectate). 5. Individuals often perform multiple preparations (washes) to extract residual drug from the filter and cooker. B, HIV may be transmitted when an HIV+ person (person 1) performs a second wash, whereby further water is added to the residual drug in the cooker/filter and the HIV-contaminated needle is used to stir the solution and reintroduced into the same filter; thus both the filter and residual drug in the cooker are contaminated. Person 1 will then draw and reinject the additional injectate. The IDPE may then be shared with an HIV- person (person 2), who would then be exposed after inserting their needle into the contaminated filter, aspirating a contaminated injectate, and injecting it.

excipients within HMC could prolong HIV survival within the IDPE and thus facilitating transmission; and heating the contaminated IDPE before each use could inactivate HIV and help prevent transmission.

METHODS

Experimental Design

The objectives of this study were to clarify the following: (1) the amount of residual hydromorphone in IDPE after the first injection (and thus the incentive for IDPE reuse) as well as the effects of heating the IDPE on the amount of hydromorphone available for injection; and (2) the persistence of HIV in IDPE in the presence of HMC or a major drug excipient and the efficacy of heating to inactivate HIV to prevent transmission associated with IDPE sharing. All experiments simulated the techniques for drug preparation used by local PWID (Fig. 2A) and were performed in triplicate with standard error bars displayed.

Objective 1: Residual Hydromorphone in IDPE and Effects of Heating

All experiments were performed with unused needles/syringes and IDPE equipment [ie, sterile water, cookers, and filters; (Sterifilt; Apothicom, Paris, France)] that were distributed through the local needle exchange program as part of the provincial harm reduction strategy and thus commonly used by local PWID. Fluid aspirated into the syringe is referred to as the "injectate" because it contains the fluid that would be injected by a PWID.

To quantify the amount of residual drug present in the cooker and filter after an initial injection, we replicated the drug preparation technique described by PWID (Fig. 2A):

crushing the drug, placing it in a cooker, dissolving it in water, and aspirating the solution through a filter. We performed this experiment with HMC capsules (24 mg; Purdue Pharma Canada, Pickering, ON) and hydromorphone immediate-release (HMI) tablets (8 mg; Pharmascience, Inc., Montreal, QC) because these are the doses and preparations identified by local addiction counselors and patient direct reports as those most commonly used locally by PWID. The contents of each HMC capsule or HMI tablet were ground in a pill crusher (Life Brand; Shoppers Drug Mart, London, ON). The ground particles were transferred into a metal cooker, and 2 mL of sterile water was added. Injectate samples were obtained by drawing the resulting mixture through a cotton filter (6 × 6-mm, tightly woven, high-density cotton; Sterifilt, Slovak Republic) with a 25-G needle (BD Canada, Mississauga, ON) into a 3-mL syringe barrel (BD Canada). This equipment is a single unit designed to reduce residual headspace. The effect of heating on HMC and HMI recovery was examined by heating the preparation with a cigarette lighter held under the cooker until the solution is boiled (ie, bubbles formed around the edge of the cooker; <10 seconds).

The drug residue remaining in the used filter and metal cooker was each assayed separately and was each combined with 3-mL methanol. Dimethylsulfoxide (SigmaAldrich, St. Louis, MO) was added at equal volume (3 mL) to methanol, and the resulting mixture was stored for 7 days at room temperature to promote additional dissolution of particles. After additional sonication (Virsonic 100; Virtis, Gardiner, NY), the mixtures were centrifuged at 14,000*g* at room temperature for 10 minutes, and the supernatants were used for drug analysis.

Hydromorphone concentrations were measured using liquid chromatography–tandem mass spectrometry. Standard curve samples were prepared by diluting hydromorphone (0–100 μg/mL final concentration; Sigma-Aldrich) in water or methanol/dimethylsulfoxide (50%/50%) for assays involving injectate or filter/residue samples, respectively. Injectate samples were diluted 500-fold with water, and an aliquot (50 μL) was combined with internal standard, alprazolam (10 μL, 10 μg/mL in methanol; ThermoFisher Diagnostix, Mississauga, ON), and 50 μL of water. After centrifugation of the sample at 14,000*g* at 22°C for 10 minutes, the resulting supernatant was diluted 20-fold with 0.1% formic acid in water. Filter/residue samples were diluted 20-fold with 0.1% formic acid in water, and then, 50 μL was combined with alprazolam (10 μL, 10 μg/mL in methanol) and 450 μL water. After centrifugation, the supernatants were diluted 20-fold with 0.1% formic acid in water.

The prepared samples were injected into the liquid chromatograph (Agilent 1200; Agilent Technologies, San Jose, CA), and analytes were separated using reverse phase chromatography on a Thermo Hypersil Gold C18 column (50 × 5 mm; 5 μm) by gradient elution. The mobile phase was delivered at a flow rate of 0.5 mL/min with gradient flow with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). For the first 60 seconds, the mobile phase consisted of 2% mobile phase B and then increased linearly to 95% mobile phase B over 3 minutes. Thereafter, a linear decrease to 2% mobile phase B occurred over 1 minute, which was held for an additional 1 minute. The retention times for hydromorphone and alprazolam were 2.9 and 4.1 minutes, respectively.

Analytes were detected by tandem mass spectrometry on a Thermo TSQ Vantage triple quadrupole instrument (ThermoFisher, San Jose, CA) equipped with a heated electrospray ionization probe (HESI-II) set with probe voltage (3000 V), vaporizer temperature (400°C), ion transfer tube temperature (350°C), sheath gas (50 arbitrary units), and auxiliary gas (10 arbitrary units). The following multiple reaction monitoring transitions were used with detection in positive mode and collision gas (1.5 mTorr): hydromorphone (286.3 → 185.2 *m/z*) and alprazolam (309.2 → 205.2 *m/z*) with collision energy at 34 and 44 V, respectively.

Objective 2: HIV Persistence in IDPE and Effects of Heating

HMC capsules and HMI tablets were prepared as mentioned previously (Fig. 2A). Two controls were used for each experiment: (1) 250 mg of the excipient microcrystalline cellulose (Sigma-Aldrich) was added to the cookers; or (2) the cookers remained empty. HIV-1 (CXCR4 tropic genotype #1389) at a stock titer of 1×10^8 viral particles per mL was diluted 1:5 in sterile water (Sigma-Aldrich), and 1.5 mL was added to each cooker. This corresponds to approximately 1–5 × 10^3 infectious units.[27] Microcrystalline cellulose was chosen due to its prevalence as an excipient, including a major component of the HMC formulation.

After 5 minutes of room temperature incubation, the solution was aspirated through the filter with a syringe (sans needle per containment laboratory requirements). To replicate reuse at different time points, this injectate preparation protocol was repeated with serial washes at 1, 4, 24, 48, and 72 hours for a total of 6 preparations for each filter. Filters were collected after the final aspiration. The insoluble residual material from the HMC and microcrystalline cellulose cookers was collected after the 24-, 48-, and 72-hour time points (50 mg per time point). To measure HIV viral persistence, injectates were split into aliquots for HIV reverse transcriptase and infectivity assays using Tzm-bl cells. Tzm-bl cells are highly susceptible to HIV infection and were used to quantify viral infectivity in the injectate samples under conditions used by PWID. Protocols for these assays have been previously published.[24] To assess the effects of heating on HIV persistence, cookers were heated with an ethanol flame until it reached its boiling point (ie, bubbling around the edge of the cooker, same as previous; <10 seconds), and Tzm-bl assays were conducted. Multiple rounds of heating were performed to investigate the reduction in infectious HIV particles.

All experiments with replication competent HIV-1 were performed in a CSL2+ facility with appropriate safety and waste protocols. HIV reverse transcriptase assays, measuring the activity of reverse transcriptase to synthesize a radiolabeled template, were measured on a STORM 860 Phosphorimager (GE Healthcare, Chicago, IL) after 24 hours of exposure on a phosphor screen. Tzm-bl cell assays,

measuring the luciferase activity produced from an HIV responsive promoter, were measured using a Britelite Plus luciferase kit (PerkinElmer, Waltham, MA) and read on a Cytation 5 Plate Reader (BioTek Instruments, Winooski, VT). All experiments were repeated with an alternate strains of HIV-1 (R5 tropic strain #147 at stock concentrations diluted 1:5, 1:10, and 1:20) and lower titers of X4 #1389 virus (stocks diluted 1:10 and 1:20).

RESULTS

Objective 1: Residual Hydromorphone in IDPE and Effects of Heating

Forty-five percent of the total hydromorphone in the HMC capsule remained in the IDPE after the first aspiration and was available for use in a subsequent wash, whereas only 16% remained in the IDPE when using HMI tablets (Fig. 3). Heating during the injection preparation did not significantly change the amount of hydromorphone in the solution within the syringe after aspiration or the amount recovered from the IDPE (Fig. 3). The preparation reached its boiling point in less than 10 seconds when using a cigarette lighter.

Objective 2: HIV Persistence in IDPE and Effects of Heating

Simulating drug preparation habits (Fig. 2A) with a consistent inoculum (~2–4 × 10^7 viral particles or ~1–5 × 10^3 IU[27]) of HIV-1 added to the IDPE allowed us to quantify relative viral persistence in different IDPE environments (Fig. 4). When preparing the HMC for injection, infectious HIV was recovered from the 5-minute, 1-, and 4- hour time points, which represented 3 separate washes of HIV-inoculated IDPE (Fig. 4A).

Significantly less infectious HIV was recovered from HIV-inoculated IDPE when preparing HMI compared with HMC (Fig. 4A). Microcrystalline cellulose is a common filler/stabilizer in controlled-release drug formulations including HMC and led to more HIV persistence than sterile water control or HMI, but less than HMC (Figs. 4A, B).

In the presence of HMC, and to a lesser extent microcrystalline cellulose, HIV reverse transcriptase activity persisted in the IDPE and was present in the fluid aspirated into the syringe (in the injectates) despite a delay of 72 hours after inoculation and 6 serial washes. HIV reverse transcriptase activity in the injectates was significantly reduced in the same conditions when originally mixed with HMI or water (Fig. 4B). These experiments were repeated at lower infectious titers (500–2500 and 250–1250 IU) and with other strains of HIV-1 (R5 strains #147). The R5 tropic strain of HIV and lower titers of all HIV-1 strains only showed detectable virus by using reverse transcriptase at the 5-minute and 1-hour time points (see Figure 1, Supplemental Digital Content, http://links.lww.com/QAI/B316). All future time points in these experiments were negative. Lower concentrations of X4-tropic HIV #1389 were also negative after the 5-minute time point.

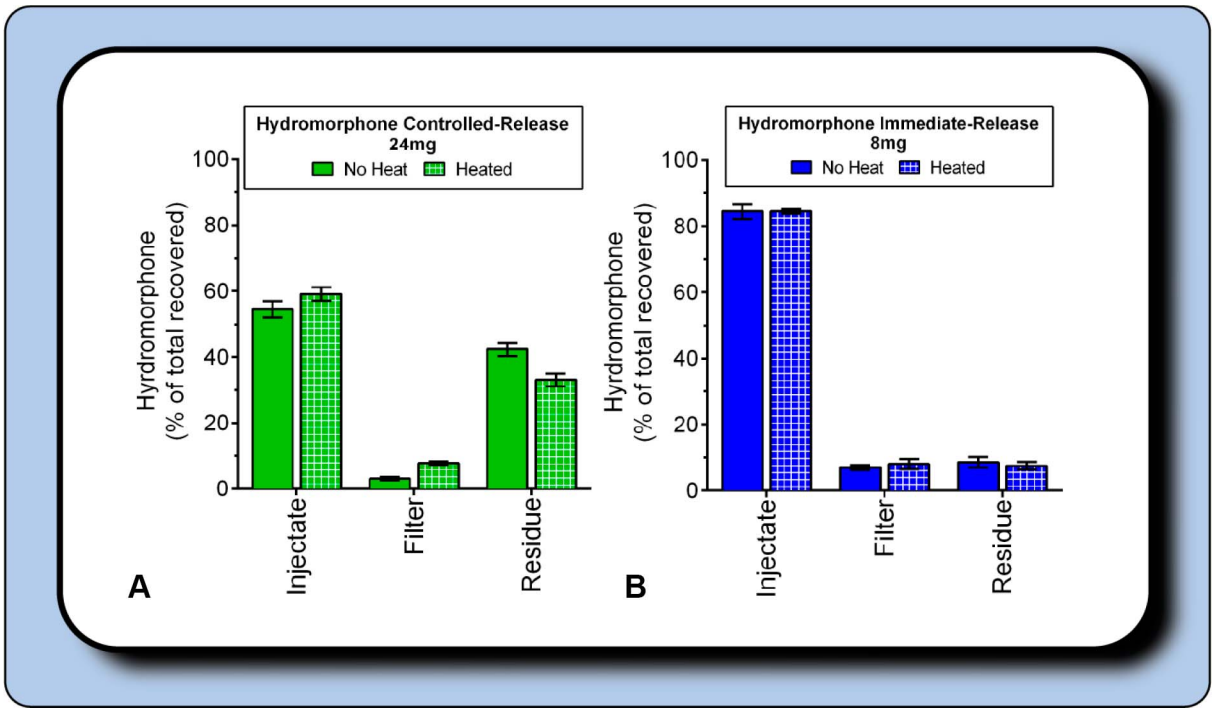

FIGURE 3. Relative amount of hydromorphone recovered in the syringe (injectate sample) and injection drug preparation equipment [filter and residue (left in cooker)] with and without heating. A, Hydromorphone controlled-release 24 mg and (B) hydromorphone immediate-release 8 mg; doses used were those most commonly injected locally. After preparation of the drug mixtures using the technique commonly used by persons who inject drugs, hydromorphone concentrations were measured using liquid chromatography–tandem mass spectrometry. Error bars represent the standard error.

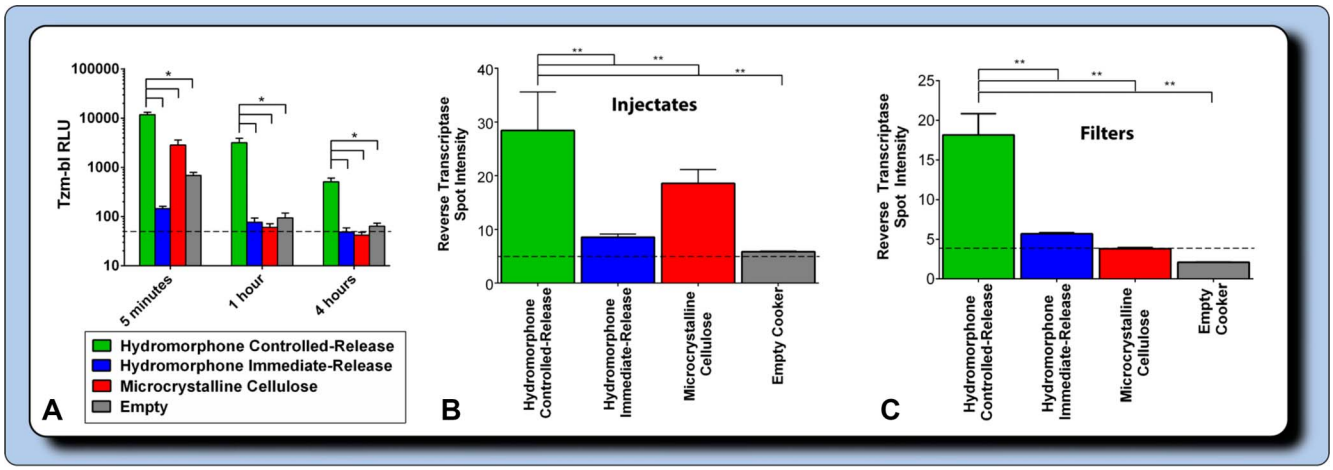

FIGURE 4. HIV infectivity and persistence after addition to injection drug preparation equipment. A, Infectivity of HIV-1 in injectates was determined by Tzm-bl cell luciferase production. HIV-1 was inoculated into cookers containing hydromorphone controlled-release or immediate-release, microcrystalline cellulose, or empty cookers to measure viral persistence. Serial washes with sterile water were collected 5 minutes, 1, and 4 hours after addition of virus. Maximal preservation of infectivity over time was seen with hydromorphone controlled-release across all measured time points. B, HIV-1 was added into cookers with varying opioid or drug excipient preparations to measure viral HIV-1 in the injectate solution. HIV-1 reverse transcriptase activity was assessed after 72 hours in the injectate solution (after 6 serial washes of the IDPE). Hydromorphone controlled-release and microcrystalline cellulose showed the greatest reverse transcriptase activity at 72 hours. C, The same method as (B) was used to measure viral HIV-1 in the filter after 72 hours. In the presence of hydromorphone controlled-release, HIV-1 reverse transcriptase activity is significantly greater than hydromorphone immediate-release. It is below the level of detection in the presence of microcrystalline cellulose and water. Dashed lines represent assay detection limits. Error bars represent the standard error. *Significant at $P < 0.05$, **Significant at $P < 0.01$ by 2-way ANOVA with the Tukey correction for multiple comparisons. ANOVA, analysis of variance.

There were some visible differences between pure microcrystalline cellulose and the excipients inside the HMC capsule. HMC, when emptied, resembles small beads that have a foam-like resistance compared with the powdered pure cellulose. These differences, and potentially the other ingredients in HMC, seem to allow for the filter to retain HIV when impregnated with HMC. Microcrystalline cellulose retains HIV in the drug residue itself but does not impregnate the filter and thus, on its own, may not result in persistence of infectious HIV. To explore these differences further, we attempted, but failed, to obtain the complete formulation of the HMC capsule without the active opioid from Purdue Pharmaceuticals.

To identify whether HIV was retained in the filters (where the needle tip of a second user would be placed), we measured reverse transcriptase activity in the filters remaining in the cooker after 72 hours. Higher levels of reverse transcriptase activity (ie, HIV) were observed in the filters in the presence of HMC compared with the other compounds (Fig. 4C). Heating the cookers with the HMC solution to a boil reduced the residual infectious HIV-1 titers in the injectate by 2 logs but did not eliminate infectious HIV. A second round of heating to a boil reduced the HIV-1 titers to the limits of detection (dotted line represents the virus-free control, Fig. 5). Further rounds of heating were performed but demonstrated no further reduction in the infectious HIV titer.

DISCUSSION

These results provide the first evidence that 45% of the contents from an HMC capsule remain in the IDPE after an initial use, thereby incentivizing the behavior of frequent IDPE reuse to access retained HMC. The current local street value of a capsule of HMC is approximately $60 CAD ($45 USD), and therefore, disposal of the remaining drug in the IDPE (a $27 CAD value) is very uncommon. This leads to IDPE being commonly shared and sold among PWIDs, despite nearly absent sharing of needles and syringes.[10] By contrast, the low quantity of residual HMI on the IDPE, because of the lower dose and higher solubility of immediate-release opioids, provides less incentive to reuse the IDPE. These findings likely explain the common behavior of multiple repeated uses of IDPE and the sharing of washes with HMC but not HMI.[10,21]

We demonstrated prolonged HIV survival in IDPE used in the preparation of controlled-release opioid (up to 3 days) compared with that used for an immediate-release opioid preparation. Although it has been demonstrated that HIV RNA can be found in high titer in IDPE used by PWID, the specific drugs used and their relative abilities to preserve HIV were not described in these studies.[22,23] It has been shown that HIV survival is reduced in the presence of heroin, but it is unclear whether this was related to the heating process required for home production or the many caustic chemicals added to produce the solution.[25] To the best of our knowledge, this study is the first to assess HIV persistence in the presence of prescribed opioids that do not require heating or the addition of extra chemicals for solubility.

Because PWID commonly reinsert their own used needle into the IDPE from multiple washes using a single needle/syringe,[10] this can lead to the IDPE becoming

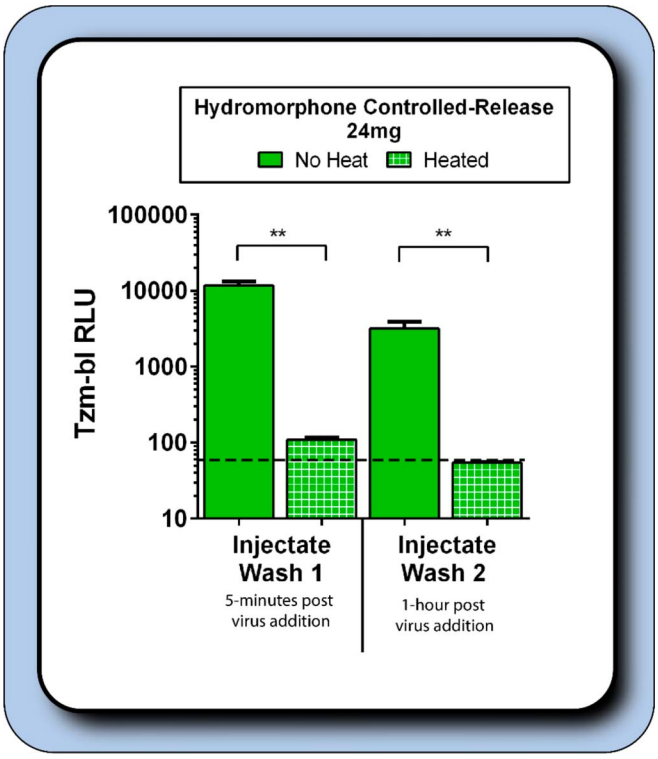

FIGURE 5. Reduction in infectious HIV-1 (measured by Tzm-bl cell luciferase production) after <10 seconds heat treatment. HIV-1 was added to cookers containing 24 mg of hydromorphone controlled-release and a cotton filter and washed with sterile water. Serial washes 1 and 2 were collected 5 minutes and 1 hour after HIV-1 addition, respectively. Cookers were then heated above a flame for <10 seconds (until bubbling) or left at room temperature during each wash. Persisting infectious HIV-1 in the injectate (fluid aspirated into a syringe for injection) was quantified by luciferase production from Tzm-bl cells. Dashed line represents the assay's detection limit. Error bars represent the standard error. **Significant at $P < 0.001$ by using 2-tailed *t* test.

contaminated with HIV. When a second PWID inserts their needle into the same filter, they can become infected through injecting. Although most PWID are careful not to share needles/syringes, they commonly reuse their own needles/syringes because they are unaware that it may contaminate the IDPE and thus increase the risk of HIV transmission through this mechanism.[11] Our demonstration that HIV can be detected not only in the injectate aspirated into the syringe from the used IDPE but also in the filter into which the second needle would be inserted suggests that contamination of the second PWID's injection would be very feasible (Figs. 2B and 4).

HIV persistence may be related to excipients in the controlled-release preparation because persistence was not seen with HMI tablets. Specifically, excipients in HMC that are not in HMI include ethyl cellulose, hydroxypropyl methylcellulose, and microcrystalline cellulose.[26] Addition of microcrystalline cellulose alone to the IDPE retained more viable HIV and for longer than IDPE when used with water or with HMI. Cellulose-containing drugs may maintain hydration, resulting in virus preservation. In fact, methylcellulose derivatives are a major constituent in media to maintain hydration and limit diffusion for viral plaque assays used for clinical diagnostics.[26] For pharmaceuticals with this agent that may be diverted for injection (eg, opioids), industry should consider seeking alternative excipients given the potential increased risk of bloodborne pathogen transmission.

The limiting sensitivity of the Tzm-bl assay prevented us from measuring infectivity beyond 4 hours (ie, third wash). However, previous research has indicated that reverse transcriptase activity in HIV-1 is a strong predictor of virus infectivity and has greater sensitivity/specificity than other surrogate assays such as p24 ELISAs or viral RNA load.[27]

We demonstrated that heating IDPE until boiling ("cooking the wash") reduced the amount of virus in the injectate to the limit of detection after 2 rounds of heating (Fig. 5). This process was found to be very easily performed, with boiling of the HMC solution occurring in less than 10 seconds when using a cigarette lighter; this demonstrated feasibility for widespread use by PWID and is locally referred to as "cooking your wash". The campaign emphasized the importance of cooking each wash to get the viral and bacterial[28] reduction benefits of serial boiling. Cooking the wash did not significantly increase the amount of hydromorphone in the injectate, and thus, should not lead to accidental overdose. And, it did not significantly reduce the amount of drug obtained, and so PWID should find it to be an acceptable harm reduction technique.

Using a sterile (ie, previously unused) needle for every aspirate of a drug is another useful strategy in preventing viral contamination of IDPE, but this alone will not reduce the risk of bacterial contamination of the injectate from repeated handling; boiling is necessary to reduce this potential bacterial load.[28] Boiling until there are bubbles present (for <10 seconds) as performed in this study did markedly reduce the burden of bacterial contamination,[28] in another recent study which we reported, and so this degree of boiling was used in this study and in the public health campaign to have a comprehensive intervention that could reduce both bacterial and viral risks. In vitro studies using alternative boiling durations and other pathogens may be helpful to optimize this intervention. Cleaning the IDPE with bleach before reuse has been occasionally recommended as another strategy, although it would not be acceptable in the context of retained drug and injectate being exposed to the caustic effects of bleach. Given that the formulation of HMC is a specific risk factor for reuse, the provision of injectable hydromorphone should also be considered, as per the SALOME trials.[29]

Based on the results of the current study, a "cook your wash" public education campaign was initiated in June 2017. The campaign recommended PWID to heat their IDPE with a cigarette lighter until bubbling for each wash before aspiration. Preliminary data showed a decrease in new HIV diagnoses in the second half of 2017, such that the HIV incidence for 2017 was no longer significantly above the provincial rate (Fig. 1). Anecdotal evidence suggests widespread uptake of the recommendation by PWID, but further studies are ongoing to document the changes in behavior associated with the campaign.

A limitation of this study is that we were unable to get access to used IDPE that would be suitable for testing for replication competent virus. This was because there was a strong effort to place all HIV patients on antiretrovirals immediately after diagnosis as per recent recommendations. Therefore, we did not have access to IDPE, which had been used by patients with high plasma viral loads. This meant that it was very unlikely that we would detect replication competent virus in the IDPE as this would have been difficult to do even with plasma.

The recent outbreaks of HIV in PWID emphasize the need for understanding all mechanisms of transmission. London had an outbreak of HIV despite having excellent pre-existing traditional HIV prevention programs for PWID. The outbreak was facilitated by the dual effect of a controlled-release opioid, which not only increases HIV survival in IDPE but also provides an incentive for sharing IDPE because of drug retention. These 2 effects seem to be synergistic in facilitating HIV transmission. Preventing IDPE sharing will be difficult due to the retention of valued drug in the IDPE after use, but promoting the heating of IDPE before each use may be an effective approach to reduce HIV infection. As the popularity of injecting prescription opioids continues to increase,[2,10] the implementation of harm reduction strategies that target IDPE sharing as a mechanism of HIV transmission will become increasingly important.

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REFERENCES

1. Zibbell JE, Asher AK, Patel RC, et al. Increases in acute hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. *Am J Public Health.* 2018;108: 175–181.
2. Jones CM, Christensen A, Gladden RM. Increases in prescription opioid injection abuse among treatment admissions in the United States, 2004–2013. *Drug Alcohol Depend.* 2017;176:89–95.
3. Conrad C, Bradley HM, Broz D, et al. Community outbreak of HIV infection linked to injection drug use of oxymorphone—Indiana, 2015. *MMWR.* 2015;64:443–444.
4. Taylor S. *"A State of Emergency": HIV Rates in Sask. Rise Again in 2016—Saskatchewan—CBC News*. CBC News Saskatchewan; 2017. Available at: http://www.cbc.ca/news/canada/saskatchewan/hiv-rates-rise-saskatchewan-1.4101145. Accessed December 29, 2017.
5. Lyss S, Zhang T, Oster A. HIV diagnoses among people who inject drugs—United States, 2010–2016 [abstract 970]. Program and abstracts of the Conference on Retroviruses and Opportunistic Infections, Boston, MA; March 4–7, 2018. Vol Abstract 9.
6. Mackie C. *Persons Who Inject Drugs in Middlesex-London: An Update. Report No. 040-16*. London, ON; 2016. Available at: https://www.healthunit.com/uploads/2016-06-16-report-040-16.pdf. Accessed April 24, 2019.
7. Des Jarlais DC, Friedman SR. HIV infection among persons who inject illicit drugs: problems and prospects. *J Acquir Immune Defic Syndr.* 1988;1:267–273.
8. Hillmer M, Wong A. Ontario narcotics atlas about the health analytics branch. *Ontario Narcotics Atlas Heal Anal Branch.* 2016. Available at: http://www.hpepublichealth.ca/sites/default/files/ON Narcotics Atlas FINAL %28December 2016%29.pdf. Accessed December 29, 2017.
9. Mitra S, Rachlis B, Scheim A, et al. Acceptability and design preferences of supervised injection services among people who inject drugs in a mid-sized Canadian City. *Harm Reduct J.* 2017;14:46.
10. Roy É, Arruda N, Bourgois P. The growing popularity of prescription opioid injection in downtown Montréal: new challenges for harm reduction. *Subst Use Misuse.* 2011;46:1142–1150.
11. Roy É, Arruda N, Bertrand K, et al. Prevalence and correlates of prescription opioid residue injection. *Drug Alcohol Depend.* 2016;166:69–74.
12. Chaisson RE, Bacchetti P, Osmond D, et al. Cocaine use and HIV infection in intravenous drug users in san francisco. *JAMA.* 1989;261: 561–565.
13. Zibbell JE, Hart-Malloy R, Barry J, et al. Risk factors for HCV infection among young adults in rural New York who inject prescription opioid analgesics. *Am J Public Health.* 2014;104:2226–2232.
14. Abadie R, Welch-Lazoritz M, Gelpi-Acosta C, et al. Understanding differences in HIV/HCV prevalence according to differentiated risk behaviors in a sample of PWID in rural Puerto Rico. *Harm Reduct J.* 2016;13:10.
15. Broz D, Wejnert C, Pham HT, et al. HIV infection and risk, prevention, and testing behaviors among injecting drug users—National HIV Behavioral Surveillance System, 20 U.S. cities, 2009. *MMWR Surveill Summ.* 2014;63:1–51.
16. McCoy CB, Metsch LR, Chitwood DD, et al. Parenteral transmission of HIV among injection drug users: assessing the frequency of multiperson use of needles, syringes, cookers, cotton, and water. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1998;18(suppl 1):S25–S29.
17. Koester S, Glanz J, Barón A. Drug sharing among heroin networks: implications for HIV and hepatitis B and C prevention. *AIDS Behav.* 2005;9:27–39.
18. Corson S, Greenhalgh D, Taylor A, et al. Modelling the prevalence of HCV amongst people who inject drugs: an investigation into the risks associated with injecting paraphernalia sharing. *Drug Alcohol Depend.* 2013;133:172–179.
19. Roy É, Arruda N, Leclerc P, et al. Injection of drug residue as a potential risk factor for HCV acquisition among Montréal young injection drug users. *Drug Alcohol Depend.* 2012;126:246–250.
20. Hagan H, Thiede H, Weiss NS, et al. Sharing of drug preparation equipment as a risk factor for hepatitis C. *Am J Public Health.* 2001;91:42–46.
21. Koivu S, Ball L, Gupta K, et al. An outbreak of HIV associated with sharing opiate injection preparation equipment. In. *Conference on Retroviruses and Opportunistic Infections (CROI): Abstract #963*. 2018. Available at: http://www.croiconference.org/sites/default/files/posters-2018/1430_Silverman_963.pdf. Accessed April 24, 2019.
22. Shah SM, Shapshak P, Rivers JE, et al. Detection of HIV-1 DNA in needle/syringes, paraphernalia, and washes from shooting galleries in Miami: a preliminary laboratory report. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1996;11:301–306.
23. Page JB, Shapshak P, Duran EM, et al. Detection of HIV-1 in injection paraphernalia: risk in an era of heightened awareness. *AIDS Patient Care STDS.* 2006;20:576–585.
24. Li M, Gao F, Mascola JR, et al. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol.* 2005;79: 10108–10125.
25. Pharma P. *Hydromorph Contin Product Monograph*. Pickering, ON: Purdue Pharma.
26. Baer A, Kehn-Hall K. Viral concentration determination through plaque assays: using traditional and novel overlay systems. *J Vis Exp.* 2014;93: e52065.
27. Marozsan AJ, Fraundorf E, Abraha A, et al. Relationships between infectious titer, capsid protein levels, and reverse transcriptase activities of diverse human immunodeficiency virus type 1 isolates. *J Virol.* 2004; 78:11130–11141.
28. Kasper K, Hallam B, Coleman CE, et al. Staphylococcus aureus poses a significant health concern for IV drug user populations due to the increased bacterial survival in controlled-released Hydromorphcontin [abstract II 079]. In: Program and abstracts of the 68th Annual Conference of the Canadian Society for Microbiology (Winnipeg), June 18, 2018, Ottawa, ON: Canadian Society of Microbiology.
29. Oviedo-Joekes E, Marchand K, Lock K, et al. The SALOME study: recruitment experiences in a clinical trial offering injectable diacetylmorphine and hydromorphone for opioid dependency. *Subst Abuse Treat Prev Pol.* 2015;10:3.