Human Immunodeficiency Virus Type 1 in Illicit-Drug Solutions Used Intravenously Retains Infectivity

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The stability of the human immunodeficiency virus type 1 (HIV-1) strain IIIB in drug solutions was studied. The data demonstrate that HIV-1 infectivity can be retained in drug solutions (e.g., heroin, “Khanka,” and “Vint”) for long periods of time. This fact must be taken into account when designing health education programs for the prevention of HIV and AIDS in Eastern Europe.

Since 1995, intravenous drug abuse has become the main risk factor for human immunodeficiency virus type 1 (HIV-1) infection in the majority of Eastern European countries. As of 1 November 2004, in the Russian Federation alone, 299,226 HIV-1 cases had officially been registered, more than 80% of which were associated with injection drug use (<http://www.afew.org>). Drug abuse in Eastern Europe is characterized by widespread use of homemade drugs in solution, such as “Khanka” (an opiate produced from poppy straw) and “Vint” (a stimulant-type drug produced domestically from ephedrine and containing methamphetamine). Moreover, even heroin, which is traditionally sold in its crystalline form in the West, is available on the Russian black market mostly as a ready-to-use solution in preloaded syringes. This practice of the distribution of drugs in solution form, rather than in crystalline form, can lead to large batches of drugs becoming contaminated with HIV-1. Contamination can occur either when ready-to-use solution is packaged into syringes for the retail trade on the streets or when several injectors take a drug from a common solution using their own (potentially infected) syringes. Furthermore, epidemiological data show that whole blood may be added to a freshly prepared drug solution to estimate its quality by hemolysis of erythrocytes (an indirect measure of drug concentration) (2). The potential risk of HIV-1 infection is therefore higher when injectors buy prefilled syringes with drugs in solution. Moreover, needle exchange schemes may have less effect if the drug solution itself is contaminated by HIV-1. This phenomenon of selling drugs in solution raises a question concerning the survival of HIV-1 activity in these drug solutions. In this study, we estimated the stability of the HIV-1 strain IIIB in drug solutions that are generally used intravenously in Russia.

Crystalline heroin and solutions of Khanka were obtained from the offices of the Ministry of the Interior of the Russian Federation in Irkutsk (eastern Siberia) and Krasnokamsk

(Perm region, Urals). Heroin powder was dissolved to a concentration of 50 mg/ml in distilled water and incubated for 40 min at 45°C until it was fully dissolved immediately before use in cell culture experiments. Two independently obtained batches of heroin powder were used in this study. Khanka is a liquid drug sold in disposable, ready-to-use syringes. To prepare the Khanka, poppy straw was covered with water and heated over a flame for about 15 min, and then soda and industrial solvent were added sequentially. The poppy straw was then removed, and the solvent was burned off. The remaining liquid was mixed with vinegar, and the excess water was boiled off. Acetic anhydride was added to “clean” the opium mixture. Vint was prepared from a commercial cough syrup (Solutan) containing ephedrine by use of the routine (illicit) method. Briefly, the alcohol in the cough syrup was burned off, and the remainder was mixed with sodium hydroxide and gasoline, which acts as a solvent. Sulfuric acid was then added to precipitate the drug, with red phosphorus and iodine crystals acting as the catalysts. Vint is highly acidic (pH 1.5), and the batch was neutralized with sodium bicarbonate before usage; however, the solution is extremely unstable and rapidly yields toxic products that have yet to be identified. For these reasons, neutralized Vint (pH 7.0) was used within 30 min. Nonneutralized Vint (pH 1.5) can be stored for up to 24 h at 4°C before neutralization and use. Both neutralized and nonneutralized Vint were prepared immediately before usage in cell culture. All drug solutions were filtered through a 0.45- μ m-pore-size membrane just before use.

MT-4, a human T lymphoid cell line, was obtained from the Medical Research Council AIDS Reagent Project and was grown in RPMI medium (Life Technologies Ltd., Paisley, United Kingdom) supplemented with 10% fetal calf serum, glutamine, and antibiotics. HIV-1 IIIB stock (kindly provided by R. Gallo) was prepared by collecting the culture supernatant from infected MT-4 cells, filtering the supernatant through a 0.45- μ m-pore-size membrane, and storing the filtrate in aliquots in liquid nitrogen until used. Viral activity was estimated by infecting triplicate samples of MT-4 cells with serial 10-fold dilutions of the viral supernatant. After 7 days,

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TABLE 1. HIV-1 stability in drug solutions^a

Drug	Temp (°C)	HIV-1 log TCID ₅₀ (μg/ml)	HIV-1 log TCID ₅₀ (μg/ml) after incubation with drug in solution for:						
			0 h	0.5 h	20 h	3 days	8 days	14 days	20 days
Heroin, batch 1	22	7.3	5.7	ND	5.3	4.0	3.0	ND	1.4
Khanka	22	7.3	6.0	ND	3.7	3.2	2.5	—	ND
Vint (pH 7.0)	22	7.3	7.2	7.1	ND	ND	ND	ND	ND
Vint (pH 1.5)	4	7.3	7.3	ND	2.8	ND	ND	ND	ND
Vint (pH 1.5)	22	7.3	7.0	3.4	ND	ND	ND	ND	ND
Control (dH ₂ O)	22	7.3	6.8	ND	6.7	5.1	4.1	ND	1.6
Control (RPMI 1640)	22	7.3	7.3	ND	7.0	6.0	4.7	ND	2.0
Heroin, batch 1	22	5.8	5.2	ND	4.8	3.6	—	ND	—
Heroin, batch 2	22	5.8	5.2	ND	4.2	3.6	—	ND	—
Control (dH ₂ O)	22	5.8	5.8	ND	5.5	4.3	3.8	ND	—

^a Two different batches of heroin powder were used as described in the text. The means of log TCID₅₀s from three parallel experiments are presented. —, HIV-1 infectivity was not detected; ND, HIV-1 infectivity was not determined; dH₂O, distilled water.

the infected cells were monitored for cytopathic effect. Determination of the 50% tissue culture infectious dose (TCID₅₀) was based on the Reed-Muench accumulative titration method (1). In this study, we used two preparations of HIV-1 IIIB containing 10^{7.3} and 10^{5.8} TCID₅₀s per ml.

To estimate the 50% cytotoxic dose (TD₅₀) of the illicit drugs, the MT-4 cells were cultured in the presence of different concentrations of each drug solution. After 3 to 4 days of incubation, a cytopathic effect was detected using the trypan blue exclusion method (5). The TD₅₀ was reached at heroin concentrations of 310 and 390 μg/ml for batches 1 and 2, respectively (130-fold and 160-fold dilutions of the stock solution used for injecting by intravenous-drug users [IDUs]). The TD₅₀ for neutralized (pH 7.0) and nonneutralized (pH 1.5) Vint (with methamphetamine concentrations of 50 and 500 μg/ml, respectively) corresponded to 1,000- and 100-fold dilutions of the Vint stock solution used for injecting by IDUs (with a methamphetamine concentration of ≤50 mg/ml), respectively. The TD₅₀ for Khanka corresponded to a 1:170 dilution of the liquid drug used for injecting.

In order to estimate HIV-1 infectivity, a drug stock solution or distilled water used as the negative control was mixed with the equivalent volume of the HIV-1 strain IIIB virus stock in 15-ml centrifuge tubes (Corning Costar Europe, Badhoevedorp, The Netherlands), which means that the HIV-1 isolate was initially incubated in drug solutions at the concentrations equivalent to those routinely used for injecting. Samples were removed at various times after incubation with the drug solutions and then diluted at ratios of 1:50 to 1:500 to prevent the cytotoxic activity of the drugs tested. The TCID₅₀ was estimated by infecting triplicate samples of MT-4 cells with serial twofold dilutions of the samples tested. After 7 days, the infected cells were monitored for cytopathic effect. In those samples where viral activity in MT-4 cells was not detected at any dilution point after 7 days of incubation, three additional passages were carried out through the addition of fresh MT-4 cells every 3 to 4 days at a ratio of 1:5. A trypan blue exclusion assay was used to monitor cell viability (5).

Table 1 shows that HIV-1 was infectious after 20 days of

incubation at 22°C in the mixture with the final heroin concentration of 25 mg/ml (the concentration used for injecting by IDUs), and similar results were obtained for HIV-1 mixed with distilled water or RPMI 1640. The Khanka solution retained detectable HIV-1 infectivity up to day 8 at 22°C and did not contain infectious virus on day 14. The difference between these two drugs may be related to the presence of additional, unknown components in the homemade Khanka solution which may reduce HIV-1 stability. It has been shown previously that cell-free HIV cultures remained infectious for several weeks at room temperature (4).

Owing to the instability of Vint, as described above, Vint at pH 1.5 was studied at 0, 0.5, and 20 h, and Vint at pH 7.0 was studied at 0 and 0.5 h. Vint at pH 7.0 retained full HIV-1 infectivity at 0.5 h (Table 1). The Vint at pH 1.5 caused HIV-1 activity to decrease by 20 h, but infectivity was retained throughout the period when this drug could have been used for injecting (at least 30 min at 22°C and at least 20 h at 4°C).

However, the experiments described above were done at a viral titer at least 4 to 5 orders of magnitude higher than what might be expected from an accidental contamination of the recreational-drug solution. To estimate the stability of HIV-1 IIIB at lower titers, we used another viral preparation containing 10^{5.8} TCID₅₀ per ml. The data were obtained from two batches of heroin that were collected independently. The results shown in Table 1 demonstrate that, in this case, the heroin solution retained the HIV-1 IIIB infectivity throughout 3 days of incubation at room temperature. HIV-1 infectivity has not yet been detectable after 8 and 20 days of incubation in drug solution. It is necessary to note that HIV-1 infectivity in water also declines more rapidly. One can suggest that a virus at higher titers may form aggregates that might shield the virus from contact with a drug. The lower-titer HIV-1 preparation used in these experiments is still orders of magnitude more concentrated than the virus in nature, and in actual practice, HIV-1 may retain its infectivity over a period of several hours to a few days. However, even this period of time could be enough to spread the virus via large batches of liquid drugs. Moreover, previous data showed that cell-associated or blood-associated virus is more resistant to potentially disinfecting agents (3). If HIV-1 can remain infectious in a drug solution, long-distance transportation may lead to new outbreaks in distant regions.

There is a widespread myth among IDUs that Vint kills HIV-1 immediately and hence no precautions against infection with HIV-1 are necessary when this drug is abused. However, these data demonstrate that HIV-1 retains infectivity for several hours even in an acid solution. This information about Vint is important for outreach workers involved in harm reduction programs and for health education.

The data presented in this paper provide preliminary evidence that premade recreational-drug solutions may be contaminated with HIV-1 and, therefore, present a public health risk. This evidence suggests the need for further research in this field, including the study of primary HIV-1 isolates and the testing of drug solutions that are being used in the field to establish if any of the solutions contain live virus. However, the use of drug solutions bought on the street can be very dangerous from the perspective of HIV infection, and such drugs must be sterilized at high temperatures, even in cases where

they are sold in disposable, ready-to-use syringes. The stability of HIV-1 in drug solutions must therefore be taken into account when designing health education programs for the prevention of HIV and AIDS among IDUs in Eastern Europe, and an information campaign to warn IDUs of this potential danger is to be developed.

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