別紙様式第 2-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-7

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2004 年 8 月 10 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood Gregori,L. et al., Lancet, 2004;364:529-531	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
英国では，血漿分画製剤の輸注による変異性クロイツフェルト・ヤコブ病（vCJD）の感染予防措置として，1999 年に白血球除去を一律実施した。この措置は，感染性が白血球に関連しているという推測に基いたものであった。伝達性海綿状脳症（TSE）の感染性の，白血球除去による低減効果を評価する実験で，スクレイピー感染したハムスターから採取したプール血液（全血）について，市販のフィルターによる白血球除去を実施した。さらに，白血球除去後に得られた分画中の感染価を限界希釈滴定法を用いて測定した。白血球濾過による白血球除去率は 2.9 log であったが，感染血液の総 TSE 感染性は 42 %の低下にとどまった。白血球除去は，白血球を介した TSE の感染性を低減するために必要であるものの，血液に由来する感染性を完全に除去するには決して十分でないことが，この研究で判明した。	Transmission of vCJD: a crisis avoided Wilson,K. and Ricketts,M.N., Lancet, 2004;364:477-479 Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient Peden,A.H. et al., Lancet, 2004;364:527–529

報告企業の意見	今後の対応
当論文によると，白血球除去における総 TSE 伝染性低下率はわずか 42 %であり，輸血における問題点が危惧されている。弊社の血漿分画製剤は，vCJD 症例の報告が極めて少ない北米で採集されているため，vCJD 伝播の理論的リスクはかなり低い。さらに，弊社の血漿分画製剤の製造工程は，4 log を上回るプリオンを除去することが確認されている。	vCJD の理論的リスクについては血漿分画製剤の添付文書にすでに記載されており，現時点では新たな安全対策上の措置は不要と考える。引き続き関連情報の収集に努める。

2 Glatzel M, Abela E, Maissen M, Aguzzi A. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. *N Engl J Med* 2003; 349: 1812–20.

3 Head MW, Ritchie D, Smith N, et al. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: an immunohistochemical, quantitative, and biochemical study. *Am J Pathol* 2004; 164: 143–53.

4 Hilton DA, Ghani A, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004; 203: 733–39.

5 Hilton DA, Sutak J, Smith MEF, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004; 57: 300–02.

Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood

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Lancet 2004; 364: 529–31

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In 1999, the UK implemented universal leucoreduction as a precaution against transmission of variant Creutzfeldt-Jakob disease by transfusion of domestic blood or red blood cells. We aimed to assess how effectively leucoreduction reduced infectivity of transmissible spongiform encephalopathies (TSEs) in blood. 450 mL of whole blood collected and pooled from scrapie-infected hamsters was leucoreduced with a commercial filter. Blood cell concentrations were quantified, and infectivity titres measured. Blood cell recovery and white blood cell removal complied with American Association of Blood Banks standards. Leucofiltration removed 42% (SD 12) of the total TSE infectivity in endogenously infected blood. Leucoreduction is necessary for the removal of white-cell-associated TSE infectivity from blood; however, it is not, by itself, sufficient to remove all blood-borne TSE infectivity.

Transmissible spongiform encephalopathies (TSEs) are fatal CNS infections that can incubate asymptomatically for a decade or more in human beings before the appearance of clinical disease. People in the asymptomatic phase of variant Creutzfeldt-Jakob disease (vCJD) appear healthy and donate blood with the same frequency as any healthy person. Transmission of vCJD by transfusion was recently recognised in Great Britain.[1] To reduce the risk of transfusion transmission of such diseases in human beings, the UK implemented universal leucoreduction of donated blood in 1999. This measure was based on the expectation that infectivity would be associated with white blood cells.[2] However, findings in blood from infected mice and hamsters suggested otherwise; at least 40% of the infectivity was plasma-associated, suggesting that leucoreduction would not eliminate infectivity (Rohwer laboratory, unpublished).[3] Other investigations showed no loss of infectivity when small amounts of TSE-infected plasma were passed through scaled-down filters.[4] Similarly, no significant removal of abnormal prion protein was detected when units of human whole blood, spiked with a microsomal fraction from TSE-infected brain, were passed through leucoreduction filters from any of the four major suppliers.[5] Because of reservations about the relevance of these experiments, none of these findings aroused concern.

We investigated the effectiveness of leucoreduction in removal of TSE infectivity from a human-sized unit of pooled hamster blood. To ensure that the 150 hamsters needed for a 450 mL blood pool were at the same symptomatic stage of disease (wobbling gait and head bobbing) for each of two separate experiments, 400 weanling golden Syrian hamsters (Harlan, Madison, WI, USA) were inoculated intracranially with 50 µL of brain homogenate containing about 250 infectious dose$_{sc}$ (ID_{50}) of hamster-adapted scrapie-strain 263K. A low dose of infectivity was used to preclude re-isolation of the inoculum in the blood. This animal protocol was approved by the University of Maryland Institutional Animal Care and Use Committee.

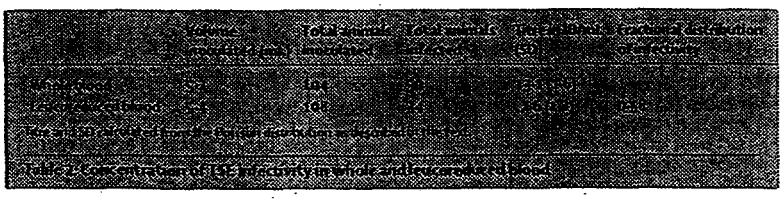
We obtained two pools of blood from the hamsters, one at 106 days and one at 111 days after inoculation. Under carbon dioxide anaesthesia, 3·5 mL of blood was drawn from the right ventricle into 0·5 mL of CP2D anticoagulant. Care was taken not to touch any other tissue. Only perfect bleeds containing 12·5% CP2D with no visible clots were pooled.

Two in-line leucofiltration systems from Pall Corporation (Port Washington, NY, USA) were evaluated. We selected the Leukotrap WB collection set for the infectivity study because filtration and component separation of hamster blood was fully compliant with American Association of Blood Banks (AABB)[6] specifications, and required only two titrations for interpretation. The Leukotrap RC-PL system

	Volume (mL)*	White blood cells: Total (% of total)	Log$_{10}$ reduction	Red blood cells, total (% of total)	Platelets, total (% of total)
Whole blood	448·5	$2·1\times10^{9}$ (100%)	0	$3·7\times10^{12}$ (100%)	$1·4\times10^{11}$ (100%)
Leucoreduced blood	424·2	$3·0\times10^{6}$ (0·15%)	2·9	$3·6\times10^{12}$ (100%)	$1·5\times10^{11}$ (100%)
Plasma	179	$3·0\times10^{6}$ (0·02%)	3·8	0 (0%)	$1·1\times10^{9}$ (9%)
Red blood cells + AS3	305·9	$2·0\times10^{6}$ (0·15%)	3	$3·1\times10^{12}$ (86%)	1×10^{11} (71%)

*Volume measurements were obtained by weight using experimentally determined densities of whole hamster blood, 1·04 g/mL. †Values are average of at least three separate microscope determinations using a haemocytometer and by flow cytometric measurements with white cells stained with propidium iodide. AS3 is a preservative and stabiliser.

Table 1: Blood component cell numbers and volumes before and after leucoreduction



approached, but did not fully achieve all specifications; furthermore, because more than one filter is involved, more titrations would have been required to evaluate the removal of infectivity.

For the infectivity study, 448·5 mL of CP2D-anticoagulated whole hamster blood was pooled into the whole-blood receiving bag of a Leukotrap WB collection set and processed within the 8-h time limit specified by the AABB. Filtration was done at room temperature under gravity with a 60-inch pressure head on the in-line WBF2 filter, and was completed in 30 min. After removal of a 19 mL sample of the leucoreduced whole blood for subsequent testing, the remainder was centrifuged at 4150 rpm (about 5000 g) for 8 min at room temperature in a Sorvall RC-3C centrifuge. The plasma fraction was expressed into a satellite in-line bag. A preservative and stabiliser, AS3, was added to the red blood cells. Samples of the pre-filtration whole blood, post-filtration whole blood, red blood cells, and plasma were removed for analysis of cell composition and for titration in animals.

Cellular composition of the blood was assessed with a HemaVet five-part differential cell counter calibrated for hamster blood cells (Drew Scientific, Oxford, CT, USA). The residual white blood cell concentrations in the leucoreduced samples were measured by manual count and flow cytometry.

Infectivity of whole and leucoreduced blood was quantified by limiting dilution titration, a method developed in the Rohwer laboratory. The two samples were processed and inoculated separately and sequentially. Each sample of blood was sonicated with a separate sterile probe to lyse cells and disperse infectivity. It was then immediately inoculated intracranially, 50 µl at a time, into about 100 weanling golden Syrian hamsters that were deeply anaesthetised with pentobarbital. Animals were maintained for 566 days; those that contracted scrapie were killed when the clinical diagnosis was conclusive, and animals still alive at the end of the study were killed. All brains were tested for the presence of the proteinase K-resistant form of prion protein by western blot using 3F4 antibody.

The limiting dilution of an endpoint dilution titration is that at which not all of the inoculated animals become infected. At limiting dilution, the distribution of infectivity into individual inoculations is described by the Poisson distribution, where P(0)=probability of no infections at that dilution and inoculation volume, or (1−probability of infection). From the Poisson distribution $P(0)=e^{-titre}$ and titre=−ln(P[0]) expressed as ID/(inoculation volume). SD of the limiting dilution titre is the square root of the titre in ID/mL divided by the total volume inoculated in mL.

Table 1 shows the distribution of cells in each component of the scrapie-infected blood. Leucofiltration reduced the number of white blood cells by 2·9 log, thereby meeting the AABB standard. White cell contamination of the red blood cell fraction and red blood cell recovery were within AABB specifications of less than 5×10^6 and greater than 85%, respectively. Hamster platelets are not removed by the WBF2 filter, and partition with the red cells during centrifugation.

The incubation times of infections in each measurement are shown in the figure. At limiting dilution, incubation times begin at the end of the predictable dose response seen in endpoint dilution titrations (about 140 days) and rarely extend beyond 500 days. All clinical and western blot results were consistent.

The limiting dilution titre of the whole blood pool (table 2) was close to the values from titrations of similar pools of whole blood by this method (unpublished data). Leucofiltration of whole blood removed only 42% (SD 12) of the initial TSE infectivity (table 2); of the 5900 ID present in the original unit of blood, 3400 ID were recovered in the leucofiltered blood.

Ideally, leucoreduction would be validated by measuring infectivity concentrations before and after leucoreduction of full units of vCJD-infected human blood. However, it is not currently possible to assay

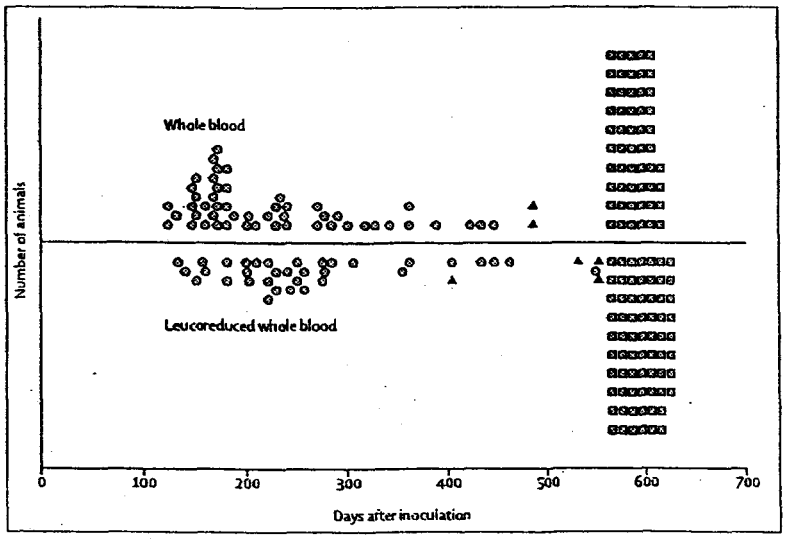


Figure: **Incubation times of infections from whole and leucoreduced blood**
Results of inoculations of whole blood are represented by data above the horizontal line; those from inoculations of leucoreduced blood are shown below the line. Circles represent infected animals. Squares represent uninfected animals that survived to the end of the experiment. Triangles represent animals that died intercurrently of causes other than the inoculum.

either infectivity or the infection-specific form of the prion protein in human blood. By contrast, limiting dilution titration of rodent blood can detect less than 1 ID/mL of TSE infectivity and can readily show a difference of less 20% between samples. With this technique we did a study that: avoided the issue of spikes by using endogenously infected blood; avoided the question of scale by using a human-sized unit of fresh hamster blood obtained within the time limits specified for human blood; minimised the possibility of artefact by using a commercial blood collection set with integral filtration unit and a blood centre centrifuge and expressor; and achieved precision in the infectivity measurements by limiting dilution inoculation of 5 mL of each fraction. We assessed the performance of the filter by measuring the level of white blood cell reduction obtained and the cell recoveries of each component. The leucoreduction met or exceeded AABB specifications for all relevant variables.

Leucoreduction removed only 42% of the initial TSE infectivity from whole blood. This distribution is consistent with that obtained in a centrifugal separation of TSE-infected hamster whole blood, in which the buffy coat contained 70% of the total white cells but only 45% of the total whole blood infectivity (unpublished data). Both methods showed that a substantial proportion of the TSE infectivity was not associated with white cells. We have shown previously[7] that TSE infectivity is not associated with highly purified platelets, and we are currently testing purified red blood cells. We presume that the majority of blood-borne infectivity is plasma-associated.

Although leucoreduction is a necessary step for removing white-cell-associated TSE infectivity from blood, this process is insufficient to remove the risk from an infected transfusion unit. Due to the low concentration of TSE infectivity in blood and the absence of screening or inactivation alternatives, removal is an attractive strategy. However, the feasibility of removal depends upon the actual associations and distributions of TSE infectivity in blood itself, which can only be ascertained by assessment of endogenous blood-borne infectivity.

Contributors

The overall design and execution of the experiment, including management of the logistics and all the infectivity work, was by L Gregori and R G Rohwer with the assistance of the staff of the Molecular Neurovirology Laboratory. A Giulivi, N McCombie, D Palmer, and P Birch supplied expertise on blood centre operations, blood collection, component separation, leucoreduction, and quantitation of white blood cells. D Palmer and P Birch undertook and interpreted flow cytometry. S Coker supplied expertise on the use of the collection set and leucofilter.

Conflict of interest statement

R G Rohwer is a cofounder and part owner of Pathogen Removal and Diagnostics Technologies, which is developing technologies for the removal of TSE infectivity from blood and other materials. L Gregori receives contract support from Pathogen Removal and Diagnostics Technologies for studies on TSE removal. S Coker is an employee of Pall Corporation, which produces leucofilters and is developing TSE removal strategies for blood. The remaining authors declare that they have no competing financial interests.

Acknowledgments

We thank the staff of the BSL-3 animal facility at the VA Medical Center, Baltimore for their excellent animal care. This study was funded by Health Canada and by the US National Heart, Lung and Blood Institute Award # HL-63930. Health Canada participated in the study design, assisted with leucofiltration, and facilitated flow cytometry analysis. They had no role in the infectivity measurements, their analysis, or interpretation. Health Canada reviewed and approved the final submission without changes. The National Heart Lung and Blood Institute participated only as a source of funding. Pall Corporation supplied a blood centrifuge, plasma expressor, and tube sealer, and served as consultants on the use of their collection sets and filters.

References

1 Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004; 363: 417–21.

2 Williamson ML. Leucocyte depletion of blood supply—how will patients benefit? *Br J Haematol* 2000; 110: 256–72.

3 Brown P, Rohwer RG, Dunston BC, MacAuley C, Gajdusek DC, Drohan WN. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. *Transfusion* 1998; 38: 810–16.

4 Brown P, Cervenakova L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. *Transfusion* 1999; 39: 1169–78.

5 Prowse CV, Bailey A. Validation of prion removal by leucocyte-depleting filters: a cautionary tale. *Vox Sang* 2000; 79: 248.

6 AABB blood bank operational manual. Standards for blood banks and transfusion services. 20th edn. Basel: Karger AG, 1998: 24–35.

7 Holada K, Vostal JG, Theisen PW, MacAuley C, Gregori L, Rohwer RG. Scrapie infectivity in hamster blood is not associated with platelets. *J Virol* 2002; 76: 4649–50.

別紙様式第 2-1

番号 36

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
一般的名称	インターフェロン α-2b (遺伝子組換え)		研究報告の公表状況	Clin. Infect. Dis. 38(9); e87-91 2004	公表国 米国	
販売名(企業名)	イントロン A(シェリング・プラウ(株))					

研究報告の概要	使用上の注意記載状況・その他参考事項等
ブラックタールヘロイン使用者間の *Clostridium sordellii* による壊死性筋膜炎の発生: カリフォルニアでは、静注薬物濫用者(IDUs)間でブラックタールヘロイン(BTH)使用により、ボツリヌス菌(*Clostridium botulinum*)による創傷ボツリヌス中毒や、破傷風菌(*Clostridium tetani*)による破傷風、種々のクロストリジウム属の菌種による壊死性柔組織感染の症例数の増加が起こっている。1999 年 12 月から 2000 年 4 月までに、カリフォルニアの Ventura 地方で 9 人の IDUs が壊死性筋膜炎を発症し、4 人が死亡した。患者 6 人の創傷検体の培養から、*Clostridium sordellii* が検出された。本発生の感染源は、皮下あるいは筋内注射された汚染された BTH だと思われる。	なし

報告企業の意見	今後の対応
本報告は、薬物使用者の注射を介した感染報告であり、原材料血液による人への感染や本製品への汚染を示す報告ではなかった。	今後とも継続的な情報収集および評価検討を行う。

36

番号

MAJOR ARTICLE

Outbreak of Necrotizing Fasciitis Due to *Clostridium sordellii* among Black-Tar Heroin Users

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In California, black tar heroin (BTH) use among injection drug users (IDUs) has resulted in an increased number of cases of wound botulism due to *Clostridium botulinum*, tetanus due to *Clostridium tetani*, and necrotizing soft-tissue infections due to a variety of clostridia. From December 1999 to April 2000, nine IDUs in Ventura County, California, developed necrotizing fasciitis; 4 died. Cultures of wound specimens from 6 case patients yielded *Clostridium sordellii*. Some of the patients appeared to have the toxic shock syndrome previously reported to be characteristic of toxin-mediated *C. sordellii* infection, which is characterized by hypotension, marked leukocytosis, and hemoconcentration. The suspected source of this outbreak was contaminated BTH that was injected subcutaneously or intramuscularly ("skin popped"). This outbreak of *C. sordellii* infection serves as another example of how BTH can potentially serve as a vehicle for transmitting severe and often deadly clostridial infections, and reinforces the need to educate IDUs and clinicians about the risks associated with skin popping of BTH.

Necrotizing soft-tissue infections, including necrotizing fasciitis, are among the most severe and life-threatening infections to affect injection drug users (IDUs) [1–3]. In recent reports of necrotizing soft-tissue infections in IDUs, the anaerobic bacteria recovered have often included clostridial species, most commonly *Clostridium perfringens* and occasionally others, such as *Clostridium sordellii* [2–4]. *C. sordellii* has been reported to cause rapidly progressive myonecrosis with a fulminant shock syndrome, particularly in obstetric patients [5–7], but its role in infections among IDUs has not been well documented.

In California, the widespread illicit use of black tar heroin (BTH) among IDUs since the 1990s has led to an epidemic of wound botulism caused by *Clostridium botulinum* [8, 9], an increase in cases of tetanus caused by *Clostridium tetani* [10], and an apparent increase in the number of necrotizing soft-tissue infections caused by *Clostridium* species [2, 3, 10]. BTH is a dark and gummy form of heroin manufactured in Mexico that is less refined and cheaper than the white powder variety of heroin. BTH is frequently mixed with a variety of diluents, such as dextrose, burned cornstarch, instant coffee, and sometimes even dirt [8]; during this process, bacterial spores can be introduced into the final product.

In February 2000, a local surgery group in Ventura County (VC), California, reported to Ventura County Public Health (VCPH) that there were 7 patients with necrotizing fasciitis who required surgical debridement from December 1999 to February 2000. All 7 patients had histories of injecting BTH subcutaneously or intramuscularly ("skin popping"). At the time of this initial report, 2 patients had laboratory-confirmed *Clostridium* infections, and 1 patient had died. Here we describe the results of our investigation into this unusual outbreak of necrotizing fasciitis among IDUs, which was likely a result of a contaminated batch of BTH.

Received 15 October 2003; accepted 14 January 2004; electronically published 14 April 2004.

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Clinical Infectious Diseases 2004; 38:e87–91

1058-4838/2004/3809-00E2$15.00

PATIENTS AND METHODS

Case finding and epidemiologic review. A case was defined as illness in a person who (1) was admitted to a VC hospital with a deep-tissue infection that required extensive surgery or was examined by the VC coroner and had deep-tissue infection listed as a cause of death, (2) had a history of heroin or BTH use, and (3) had an onset of illness during the period 1 December 1999 to 1 May 2000.

VCPH requested all VC hospitals and the VC coroner's office to review their records and report all patients who received a diagnosis of necrotizing fasciitis during the 5-month period from 1 December 1999 to 1 May 2000. Once a case patient was verified as meeting the case definition, hospital records were reviewed by means of a standardized chart-abstraction form to collect demographic information, clinical history, hospital course, medications administered, laboratory tests performed, and hospital costs charged. Attempts were also made to locate and interview surviving patients regarding their medical history and drug use behaviors. For the 1 case reported by the coroner, the autopsy report and death certificate were reviewed.

Laboratory investigation. Wound specimens from hospitalized patients were cultured by the clinical laboratories at the admitting hospitals with standard aerobic and anaerobic techniques. Wound specimens from the coroner's case patient were sent to the VCPH laboratory for performance of aerobic and anaerobic cultures. In addition, the VC Sheriff's Department confiscated BTH from a heroin dealer during a drug raid in February 2000. A sample of this BTH was sent to the Centers for Disease Control and Prevention in Atlanta, Georgia, to culture for anaerobic pathogens.

Data analysis. Chart abstraction data were entered into a Microsoft Access database and analyzed with SAS, version 8.00 (SAS Institute). Because of the small number of cases, the Wilcoxon rank-sum exact test was used to compare continuous variables for patients who survived with those for patients who died.

RESULTS

Descriptive epidemiology. A total of 9 persons met the case definition; 8 were reported by VC hospitals and 1 by the VC coroner's office. The dates of illness onset ranged from 14 December 1999 to 8 April 2000. The median age of patients was 45 years (range, 25–57 years) (table 1); 6 (67%) were men. Six patients were Hispanic and 3 were white. None were known to have HIV infection, hepatitis B infection, or diabetes; however, 3 (33%) were known to have hepatitis C infection. Four patients (44%) died, 3 in hospitals and 1 (the coroner's case patient) at a friend's home. Six (67%) were known to have used BTH; the specific type of heroin used was unknown for 3 of the patients. All 8 hospitalized patients had histories of skin popping heroin before admission.

Hospitalized patients. Seven cases were reported by hospital A (a county facility) and 1 by hospital B (a community facility). Table 1 shows the vital signs at presentation for the hospitalized patients. The median time between symptom onset and hospital admission was 3.5 days (range, 2–7 days). It is notable that all patients were afebrile (median temperature, 36.4°C; range, 34.4°C–37.1°C). Blood pressures ranged from 81/49 to 142/90 mm Hg. Four patients had infection consistent with necrotizing fasciitis in the upper arm/shoulder region and 4 in the hip/buttocks/thigh region. For all 8 patients, surgical debridement was performed after admission; no patients required amputation. Two patients died within 24 h after surgery. Five patients from hospital A were transferred after surgery for

Table 1. Hospital admission characteristics of black-tar heroin users with necrotizing fasciitis, Ventura County, California, December 1999–April 2000.

Parameter	Median value (range), by patient group			
	All patients (n = 8)	Patients who survived (n = 5)	Patients who died (n = 3)	P[a]
Age, years	42.5 (25–57)	34 (25–45)	55 (47–57)	.03
No. of days from onset of symptoms to admission	3.5 (2–7)	4 (2–5)	3 (3–7)	1.00
Systolic blood pressure, mm Hg	111.5 (81–142)	113 (102–128)	110 (81–142)	1.00
Diastolic blood pressure, mm Hg	69.0 (49–90)	68 (49–86)	90 (49–90)	.50
Temperature, °C	36.4 (34.4–37.1)	36.4 (36.3–37.1)	35.6 (34.4–36.1)	.02
Pulse, beats/min	102.5 (83–142)	98 (83–140)	110 (98–142)	.29
Respiration rate, breaths/min	17 (12–40)	18 (16–20)	16 (12–40)	.66
WBC count, ×1000 cells/mm^3	28.3 (9.8–61.6)	23.2 (9.8–32.3)	54.3 (54.3–61.6)	.04
Hemoglobin level, g/dL	14.6 (11.7–22.0)	14.0 (11.7–15.1)	21.6 (19.5–22.0)	.04

[a] Wilcoxon rank-sum exact test comparing patients who survived with those who died.

hyperbaric oxygen treatment to hospital C, where 1 patient died 3 days after surgery.

The 3 hospitalized patients who died and the 5 who survived differed in some characteristics (table 1). Patients who died were older than those that survived ($P = .03$). Furthermore, at presentation, those who died had lower body temperatures ($P = .02$) and more marked leukocytosis ($P = .04$). In addition, the patients who died had higher hemoglobin levels (median of 21.6 g/dL, compared with 14.0 g/dL for survivors; $P = .04$), and all 3 had extensive involvement of the hip/buttock/thigh region. The patients who died had refractory hypotension despite administration of aggressive fluid resuscitation before death. The cause of death for all 3 hospitalized patients was overwhelming sepsis secondary to necrotizing fasciitis. However, the patients who died did not wait longer after the onset of symptoms before seeking medical treatment than did the patients who survived.

For the 3 hospitalized patients who died, the median hospital charge was $31,763 (range, $9398–$55,597), and the median length of stay before death was 3 days (range, 1–4 days). For the 5 surviving patients, the median hospital charge was $74,179 (range, $7861–$130,439), and the median length of stay was 14 days (range, 5–47 days). Total charge for all 8 hospitalized patients was $398,341. Two patients had private insurance, 1 had Medicaid coverage, 4 were uninsured, and 1 had unknown insurance status.

Coroner's case patient. The VC coroner's office reported 1 case patient who died at a friend's home ~4 days after onset of pain and redness in the left arm. The coroner's report listed "acute morphine intoxication" as the cause of death and "chronic parenteral drug abuse with acute and chronic necrotizing fasciitis of left arm" as the underlying cause of death.

Laboratory investigation. Wound specimens were collected from all 9 patients. All patients had at least 1 culture that yielded ≥1 species of bacteria. *C. sordellii* was isolated from 6 of the patients; *Staphylococcus* species (at least 2 isolates were *Staphylococcus aureus*) from 4 patients; α-hemolytic *Streptococcus* species from 3 patients; and *Bacillus* species from 2 patients (table 2). Blood cultures for all patients, including patients who died, did not yield pathogens. No organisms were recovered from the BTH sample obtained by the VC sheriff's department.

Patient interviews. VC case workers were able to locate and interview 3 of the 5 surviving patients. Two patients independently mentioned the same park in VC as their source of drugs. The third patient would not reveal his drug source. All 3 reported that they used BTH.

DISCUSSION

We suspect that a batch of BTH contaminated with *C. sordellii* was sold within VC from the end of 1999 through the early months of 2000, resulting in an outbreak of necrotizing fasciitis among IDUs. During this time, 9 IDUs in VC developed necrotizing fasciitis; 4 died. *C. sordellii* was the most commonly isolated organism, present in 67% of the patients.

C. sordellii is an anaerobic, gram-positive, spore-forming bacillus that can be isolated from environmental sources and from normal human and animal gastrointestinal contents [5]; however, *C. sordellii* is occasionally reported as the cause of fulminant infection, most commonly in obstetric patients [6, 7]. *C. sordellii* deep-tissue infection has also been associated with injection drug use. This organism was the most commonly isolated anaerobic organism (4 of 9 anaerobic isolates) in a case series of IDUs with necrotizing soft-tissue infections at a San Francisco hospital during 1992–1997 [2]. In 1999, another cluster of clostridial myonecrosis occurred among San Francisco IDUs; 2 of 5 patients had wound specimens that yielded *C. sordellii* [11]. Nonetheless, to our knowledge, our cluster of *C. sordellii* infections is the largest reported to date, and it serves as another example of how BTH can potentially serve as a

Table 2. Isolates from culture of wound specimens from black-tar heroin users with necrotizing fasciitis, Ventura County, California, December 1999–April 2000.

Patient	Date of onset of symptoms	Pathogen(s) isolated				Disposition at discharge
		Organism 1	Organism 2	Organism 3	Organism 4	
1	14 Dec 1999	*Clostridium sordellii*	*Staphylococcus* species	...	...	Alive
2	28 Jan 2000	*Bacillus* species	...	...	...	Dead
3	30 Jan 2000	α-Hemolytic *Streptococcus* species	*Staphylococcus* species	...	...	Alive
4	1 Feb 2000	*C. sordellii*	*Clostridium perfringens*	...	...	Alive
5	4 Feb 2000	*C. sordellii*	*Staphylococcus* species	...	...	Alive
6	6 Feb 2000	α-Hemolytic *Streptococcus* species	...	...	...	Alive
7	21 Feb 2000	*C. sordellii*	*C. perfringens*	*Clostridium beijerinckii*	*Bacillus* species	Dead
8	1 April 2000	*C. sordellii*	...	...	...	Dead
9	8 April 2000	*C. sordellii*	α-Hemolytic *Streptococcus*	*Staphylococcus* species	...	Dead

NOTE. Patients 1–8 were reported by hospitals; patient 9 was reported by the coroner.

vehicle for transmitting severe and often deadly clostridial infections.

Some strains of *C. sordellii* are toxigenic under certain circumstances [7]. *C. sordellii* has been described as having the potential to cause a fulminant toxic shock syndrome characterized by absence of fever, progressive refractory hypotension, tissue edema, marked leukocytosis, and hemoconcentration [7, 12]. This syndrome is thought to be mediated by 2 toxins, lethal and hemorrhagic, that are released by some strains of *C. sordellii* [5–7]. Intradermal injection of these toxins into animals results in local necrosis, progressive edema due to local and systemic vascular permeability, and death, a process similar to that seen in some *C. sordellii* infections in humans [13, 14]. It is plausible that some of our patients may have skin-popped BTH contaminated with *C. sordellii*, which led to toxin production, which in turn resulted in severe necrotizing disease, toxic shock syndrome, and death.

Injecting BTH into soft tissue rather than the bloodstream increases the risk of abscess formation, and the resultant devitalized tissue sets up an anaerobic environment conducive to clostridial toxin formation [9]. Wound botulism occurs after spores of *C. botulinum* germinate in a wound and produce botulinal toxin, resulting in flaccid paralysis [8, 9, 15]. In California, the primary risk factor for wound botulism is skin popping of BTH [8]. Similarly, tetanus develops when *C. tetani* introduced into a wound germinates and releases toxin [16]. Nearly one-half of the cases of tetanus reported in California between 1988 and 2000 were associated with injection drug use; of these, at least 60% were associated with reported heroin or BTH use [10]. We suspect that the conditions that lead to toxin production by *C. sordellii* are similar to those that lead to toxin production by *C. botulinum* and *C. tetani* [8–10].

Clostridial contamination of heroin has been documented. In Scotland, Ireland, and England in 2000, an outbreak of serious soft-tissue infections, mainly due to *Clostridium novyi*, occurred among IDUs who skin-popped heroin [17, 18]. A contaminated supply of heroin was suspected to be the source; 2 species, *C. perfringens* and *Clostridium saccharolyticum*, were isolated from heroin belonging to the patients [17]. In the 1999 San Francisco cluster, 3 of the 5 patients were roommates who shared a supply of BTH [11]. One roommate had confirmed *C. perfringens* infection, and another had *C. sordellii* infection. BTH confiscated from their apartment yielded *C. perfringens*, demonstrating that it was likely the common source of their necrotizing soft-tissue infections [11].

In our outbreak investigation, no anaerobic organisms were isolated from the sample of BTH confiscated during the drug raid; however, the BTH was not necessarily from the suspected supply that made the VC patients ill. Nonetheless, evidence suggests that BTH can be contaminated by clostridia before purchase by the user, most likely when being mixed with diluents [8, 9]. Although we cannot rule out the possibility that the patients in our study became infected through contaminated needles or other drug-injection paraphernalia, it is most likely that the contaminated BTH was the source of the necrotizing infections. This hypothesis is supported by the following evidence: (1) interviews with 3 surviving patients confirmed that they did not know each other and did not share paraphernalia but did use BTH; (2) 2 of the interviewed patients reported that they had purchased BTH at the same VC park; (3) *Clostridium* species have previously been isolated from BTH [11], so the hypothesis is biologically plausible; and (4) there is an ongoing California epidemic of infections due to 2 other toxin-producing *Clostridium* species, *C. botulinum* and *C. tetani*, also due to skin popping of contaminated BTH [8–10].

Necrotizing fasciitis requires aggressive and timely surgical debridement to prevent rapid progression of infection [19]. However, necrotizing fasciitis can sometimes be difficult to distinguish from simple cellulitis or abscess, creating a diagnostic challenge [2]. Physicians should evaluate for necrotizing fasciitis whenever an IDU presents with an infection at an injection site. It is notable that, among the cases in our cluster, marked leukocytosis, high hemoglobin count, and lower body temperature at presentation were associated with death. Although only 2 of the 3 hospitalized patients who died had cultures that yielded *C. sordellii*, all 3 seemed to develop the toxic shock syndrome reported to be characteristic of toxin-mediated *C. sordellii* infection [5–7]. Physicians should be alerted to the presence of this syndrome; survival may be improved by prompt recognition and aggressive treatment. Antitoxin treatment of this toxic shock syndrome due to *C. sordellii*, similar to the treatment given for botulism and tetanus, has been suggested [5–7]. Further research into the effectiveness of this treatment is needed.

Treatment of necrotizing fasciitis is costly because of the need for wound debridement, treatment with multiple antibiotics, hyperbaric treatments, and lengthy hospital stays. The total charge for the 8 hospitalized patients in this investigation was ~$400,000; only 2 patients had private health insurance. This imposes a large financial burden on the health care system that could potentially be reduced through aggressive education of IDUs and physicians.

Although many IDUs understand the risks of contracting hepatitis and AIDS from injecting drugs, most may be unaware of the additional and potentially lethal health risks associated with skin popping of BTH, such as the development of necrotizing fasciitis. IDUs should be instructed to seek medical attention at the first sign of infection at an injection site because of the rapid progression and high fatality rate of necrotizing fasciitis. Correctional facilities, halfway homes, methadone clinics, and other agencies caring for IDUs should be used as venues to educate IDUs about the dangers of skin popping of BTH.

Acknowledgments

We thank the following individuals for their assistance in this investigation: Ben Werner, Infectious Diseases Branch, California Department of Health Services; Susan Maslanka, National Botulism Surveillance and Reference Laboratory, Centers for Disease Control and Prevention; Elise McKee and Marilyn Billimek, Ventura County Public Health; Dr. James L. Holden and the Tower Surgical Associates Medical Group; and the Ventura County Sheriff's Department.

References

1. Haverkos H, Lange R. Serious infections other than human immunodeficiency virus among intravenous drug abusers. J Infect Dis 1990; 161:894–902.
2. Callahan T, Schecter W, Horn J. Necrotizing soft tissue infection masquerading as cutaneous abscess following illicit drug injection. Arch Surg 1998; 133:812–7.
3. Chen, JL, Fullerton KE, Flynn NM. Necrotizing fasciitis associated with injection drug use. Clin Infect Dis 2001; 33:6–15.
4. Summanen PH, Talan C, Strong M, et al. Bacteriology of skin and soft-tissue infections: comparison of infections in intravenous drug users and individuals with no history of intravenous drug use. Clin Infect Dis 1995; 20(Suppl 2):S279–82.
5. Abdulla A, Yee L. The clinical spectrum of *Clostridium sordellii* bacteraemia: two case reports and a review of the literature. J Clin Pathol 2000; 53:709–12.
6. Sinave C, Templier G, Blouin D, Leveille F, Deland E. Toxic shock syndrome due to *Clostridium sordellii:* a dramatic postpartum and post abortion disease. Clin Infect Dis 2002; 35:1441–3.
7. McGregor J, Soper D, Lovell G, Todd J. Maternal deaths associated with *Clostridium sordellii* infection. Am J Obstet Gynecol 1989; 161: 987–95.
8. Passaro DJ, Werner SB, McGee J, MacKenzie WR, Vugia DJ. Wound botulism associated with black tar heroin among injecting drug users. JAMA 1998; 279:859–63.
9. Werner SB, Passaro D, McGee J, Schechter R, Vugia DJ. Wound botulism in California, 1951–1998: recent epidemic in heroin injectors. Clin Infect Dis 2000; 31:1018–24.
10. Vugia DC, Werner SB, Woodfill CJ. Wound botulism, tetanus, and necrotizing fasciitis among injection drug users in California: the clostridial connection. In: Scheld WM, Murray BE, Hughes JM, eds. Emerging infections 6. Washington, DC: ASM Press, 2004:111-20.
11. Bangsberg D, Rosen J, Aragon T, et al. Clostridial myonecrosis cluster among injection drug users: a molecular epidemiology investigation. Arch Intern Med 2002; 162:517–25.
12. Rorbye C, Petersen I, Nilas L. Postpartum *Clostridium sordellii* infection associated with fatal toxic shock syndrome. Acta Obstet Gynecol Scand 2000; 79:1134–5.
13. Browdie D, Davis J, Koplewitz M, Corday L, Leadbetter A. *Clostridium sordellii* infection. J Trauma 1975; 15:515–8.
14. Arseculeratme SN, Panabokke RG, Wijesundera S. The toxins responsible for the lesions of *Clostridium sordellii* gas gangrene. J Med Microbiol 1969; 2:37–53.
15. Shapiro R, Hatheway C, Swerdlow D. Botulism in the United States: a clinical and epidemiologic review. Ann Intern Med 1998; 129:221–8.
16. Bleck TP. *Clostridium tetani* (tetanus). In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's principles and practice of infectious disease. 5th ed. Vol 2. Philadelphia: Churchill Livingstone, 2000:2537–43.
17. McGuigan CC, Penrice GM, Gruer L, et al. Lethal outbreak of infection with *Clostridium novyi* type A and other spore-forming organisms in Scottish injecting drug users. J Med Microbiol 2002; 51:971–7.
18. Jones JA, Salmon JE, Djuretic T, et al. An outbreak of serious illness and death among injecting drug users in England during 2000. J Med Microbiol 2002; 51:978–2.
19. Ellliott D, Kufera J, Myers R. Necrotizing soft tissue infections: risk factors for mortality and strategies for management. Ann Surg 1996; 224:672–83.