The use of a novel biodegradable preparation capable of the sustained release of bacteriophages and ciprofloxacin, in the complex treatment of multidrug-resistant *Staphylococcus aureus*-infected local radiation injuries caused by exposure to Sr90

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Summary

In December 2001, three Georgian lumberjacks from the village of Lia were exposed to a strontium-90 source from two Soviet-era radiothermal generators they found near their village. In addition to systemic effects, two of them developed severe local radiation injuries which subsequently became infected with *Staphylococcus aureus*. After hospitalization in Tbilisi, Georgia, the patients were treated with various medications, including antibiotics and topical ointments; however, wound healing was only moderately successful, and their *S. aureus* infection could not be eliminated. Approximately 1 month after hospitalization, treatment with PhagoBioDerm (a wound-healing preparation consisting of a biodegradable polymer impregnated with ciprofloxacin and bacteriophages) was initiated. Purulent drainage stopped within 2–7 days. Clinical improvement was associated with rapid (7 days) elimination of the aetiologic agent, a strain of *S. aureus* resistant to many antibiotics (including ciprofloxacin), but susceptible to the bacteriophages contained in the PhagoBioDerm preparation.

Report

Subcutaneous tissues exposed as a result of skin damage provide a favourable environment for contamination and colonization with a wide variety of microorganisms, which can present a substantial clinical problem – especially if the infecting bacteria are resistant to antibiotics.[1] In this manuscript, we report the effective use of a novel, bacteriophage-releasing, biodegradable preparation (PhagoBioDerm™) for treating two patients with severe local radiation burns infected with multidrug-resistant *Staphylococcus aureus*.

On 2 December 2001, three inhabitants (1-DN, 2-MG, and 3-MB) of the village of Lia in Georgia (one of the former Soviet Union Republics) went to collect firewood in the woods around 50 km east of their village. While working, they discovered two metallic, cylindrical objects ($\approx 10 \times 15$-cm canisters) around which the snow had melted, with steam coming from the wet soil. The canisters were later identified as Soviet-era radiothermal generators containing strontium-90.[2,1,2] However, the lumberjacks were not aware of this, and they used the two canisters to heat themselves during the cold December night. Two of the patients (1-DN and 2-MG) also reclined on the cylinders for about 1.5 h. Approximately 4 h after their first contact with the canisters, all three individuals developed

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headache, nausea and dizziness, followed by intense vomiting (more than 20 occasions). Their symptoms gradually subsided by the morning, and they returned to their village. Two of the patients (1-DN and 3-MB) did not seek immediate medical attention, attributing their illness to 'food poisoning.' The third patient (2-MG) visited his physician on day 3 post-exposure, and was treated with two intravenous infusions of saline and Haemodes (for possible intoxication), and an intramuscular injection of the antihistamine chloropheniramine (for a possible allergic reaction). His acute symptoms resolved, and he remained asymptomatic for 14 days post-exposure.

On day 14 post-exposure, patients 1-DN and 2-MG developed a burning sensation on their backs, which escalated into dry and then wet desquamation and severe pain during the next few days (delay between exposure and appearance of lesions is common after X-ray burns).[3] A diagnosis of 'acute radiation disease' was made, and all three patients were hospitalized in the Scientific Research Institute of Hematology and Transfusiology (SRIHT) in Tbilisi on 23 December 2001 (i.e., 21 days post-exposure). The diagnosis was confirmed at the SRIHT, and the patients were also found to have bone marrow suppression, with oropharyngeal and cutaneous symptoms; symptoms of patients 1-DN and 2-MG, and the limited laboratory data available for them, are summarized in Table 1. In addition, 1-DN and 2-MG developed severe local radiation injuries on their backs. Clinical manifestations were worse than those of the third patient (3-MB), possibly because they had direct contact with the radiation source as they reclined on it, whereas 3-MB used it only as a more-distant heat source. *S. aureus* was isolated from the wounds of 2-MG and 1-DN on day 7 after hospitalization. The isolates were resistant to penicillin, streptomycin, chloramphenicol, ampicillin, oxacillin, gentamicin, erythromycin, doxycycline, ciprofloxacin, rocepin (triaxon), and cefotaxime; they were moderately susceptible to gentamicin, and they were susceptible to vancomycin. Molecular typing to determine whether both patients were infected with the same *S. aureus* strain or with two different isolates was not performed; however, antibiotic susceptibility patterns suggested that the infections in the two

Table 1 Case descriptions of two patients infected with *Staphylococcus aureus* after exposure to Sr^{90}.

Patient	Sex/Age	Clinical symptoms 3-4 h post exposure	Clinical symptoms at the time of hospital admission (3 weeks post exposure)	Treatment and outcome	
				Before PhagoBioDerm™ (duration: ca. 1 month)	After PhagoBioDerm™* (single application)
1-DN	Male/45	Nausea, headache, dizziness, and intensive, long-lasting vomiting	40°C fever, severe pain in throat, low blood pressure (70/30 Hg/mm), severe leucopenia (0.1 G/L) and thrombocytopenia (8 G/L), and elevated creatinine level (250 μmol/L). Two large ulcers (infected with *S. aureus*) on the back; 14 cm and 17 to 18 cm in diameter (Fig. 1A)	IV injection of saline, rheopolyglukin, and Aminosol (to improve nutrition), and granulocyte colony-stimulating factor and concentrated preparations of platelet and erythrocytes. Antibiotics: rocepin (triaxon, 2 g) twice/day, and gentamicin (80 mg) 3 times/day. Olasolum, Panthenol, and Solcoseryl applied locally, and two doses of Diflucan (150 mg/day) to prevent fungal infection. Purulent discharge was not stopped, and *S. aureus* was consistently recovered from the infected ulcers	Significant reduction of purulent discharge in 2 days, and complete loss of purulence on day 7. *S. aureus* was eliminated from ulcers on day 7
2-MG	Male/52	Nausea, itching, headache, dizziness, diarrhoea, and intensive, long-lasting vomiting	Herpes simplex at admission. Other clinical symptoms were similar to, but somewhat milder than, those of 1-DN; also observed leucopenia (0.6 G/L) and thrombocytopenia (20 G/L). A very large (38 × 35 cm) burn on the back was infected with *S. aureus*	IV injection of saline, Haemodes, and antibiotics (triaxon). Narcotics as painkillers. Acyclovir (per os and locally) for 5 days, for herpes infection. Purulent discharge was not stopped, and *S. aureus* was consistently recovered from the infected burn wound	Significant reduction of purulent discharge in 2 days, and complete loss of purulence on day 7. *S. aureus* was eliminated from the burn wound on day 7

* Conventional treatment (as described in the "Before PhagoBioDerm™" column) continued, with the exception of the use of ointments.

Acyclovir (GlaxoSmithKline, USA): synthetic nucleoside analogue active against herpes viruses.

Haemodes (Biokhimik, Russia): low molecular weight dextran, used to eliminate toxic metabolites via renal clearance.

Olasolum (Altaivitamins, Russia): a foam-forming spray containing chloramphenicol, anesthesin, boric acid, and vegetable oils.

Solcoseryl (Alkaloid, Macedonia): a deproteinized extract of calf blood, used to promote epithelization.

Panthenol (Chauvin Ankerpharm, Germany): spray used as a biostimulator for skin regeneration (active ingredient is dexpanthenol).

patients were caused by the same or a closely related *S. aureus* strain.

After admission to the SRIHT, the patients received blood and platelet transfusion for anaemia and thrombocytopenia. In addition, they received large intravenous doses of ceftriaxone (2 g twice daily) and gentamicin (80 mg three times daily) starting on the day of admission (antibiotic therapy was continued, to prevent other possible infections, even after the infecting *S. aureus* strain was determined to be resistant to these antibiotics). Also, various ointments (including antibiotic-containing ointments) were used for the infected ulcers. More detailed information about the treatment received by the two patients is given in Table 1. However, despite aggressive antimicrobial therapy, wound healing was only moderately successful in both patients, and purulent drainage was not eliminated even after 23 days of treatment. Moreover, *S. aureus* was consistently isolated from swab samples taken from both patients' wounds. Therefore, the patients' primary physicians made the decision to use lytic bacteriophages against *S. aureus* as part of their treatment, and the use of PhagoBioDerm was initiated in both patients on 21 January 2002 (1 month after admission).

PhagoBioDerm is a relatively novel wound-healing preparation containing a biodegradable polymer matrix impregnated with a mixture of bacteriophages with lytic activity against *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus*, and *Proteus*.[4,7] The *S. aureus* strains isolated from the two patients were susceptible to the bacteriophage preparation included in PhagoBioDerm, as determined by using a previously described modified disk diffusion method[7] modelled after the standard Kirby–Bauer procedure for determining antimicrobial susceptibility. Because of the large size of the ulcers, multiple films of PhagoBioDerm were used to cover the wound surfaces (the left scapula area of 1-DN, and the clinically most affected and painful area in the centre of local radiation injury on the lower back of 2-MG). The films were immobilized with two or more sterile bandages in order to minimize their movement and to absorb exudate. The wounds were examined daily during the first 5 days after film application, and once every 2–4 days thereafter. Swab samples were taken from the wounds, and the infecting bacteria were enumerated using a standard colony counting technique.

Two days after PhagoBioDerm application, purulent drainage from both patients' ulcers decreased significantly (to almost none), and they also noted a decrease in pain in their wounds. On the seventh day of treatment, the ulcers tested negative for *S. aureus*. PhagoBioDerm application and wound healing in one patient (1-DN) are shown in Fig. 1. The patients were flown to Russia (patient 1-DN) and France (patient 2-MG) for skin grafting during February 2002, and they are currently recovering in Moscow, Russia (patient 1-DN) and in his native village, Lia (patient 2-MG).

The value of sustained drug delivery for treating wounds – including wounds infected with multidrug-resistant *S. aureus* – has been well-documented,[4,5,6,10] and our report provides further evidence of the usefulness of this approach. The three unique features of the cases described here are: (i) the novelty of the biodegradable polymer; (ii) the inclusion of bacteriophages as antimicrobial agents in a biodegradable matrix (phages have been extensively used therapeutically in different settings);[9] and (iii) the nature of the wounds. The current study was not designed to be a double-blind, placebo-controlled trial evaluating the efficacy of PhagoBioDerm for treating infected burns. Moreover, a control group could not be included because of the peculiarity of the accident and the small number of patients requiring the treatment and because of concern

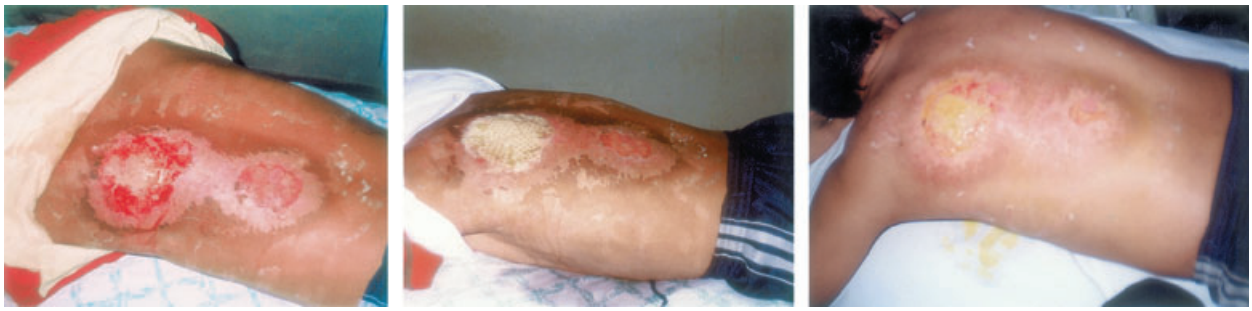

Figure 1 The use of PhagoBioDerm for wound healing in patient 1-DN. The pictures show (from left to right) the purulent lesion on day 23 of hospitalization, application of PhagoBioDerm on day 29 of hospitalization, and wound healing after 23 days.

on the part of the clinicians that continued application of conventional therapy in the patients would continue to be ineffective. Nonetheless, the fact that purulent drainage stopped and *S. aureus* was eliminated from the wounds within ≈ 7 days post-treatment with PhagoBioDerm (while the preceding 1 month of aggressive treatment with ointments and antibiotics did not achieve this result) strongly suggests that PhagoBioDerm was responsible for eliminating the infecting *S. aureus* strain from the wounds of the patients and, possibly, for the marked improvement in wound healing observed in both patients. Furthermore, the observation that the infecting strains were resistant to one (ciprofloxacin) of the two antimicrobial agents (ciprofloxacin and bacteriophages) included in PhagoBioDerm suggests that the elimination of *S. aureus* from the wounds was due to the lytic effect of bacteriophages rather than to the action of ciprofloxacin. This observation supports previously reported studies[7,8,11] that bacteriophages can be useful for treating infected wounds, and it suggests that PhagoBioDerm and similar products may provide an effective alternative means for treating infected wounds, including wounds infected with antibiotic-resistant bacteria.

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References

1 Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001; **14**: 244–69.
2 Stone R. Nuclear waste. 'Hot' legacy raises alarm in the Caucasus. *Science* 2002; **295**: 777–9.
3 Nenot JC. Medical and surgical management for localized radiation injuries. *Int J Radiat Biol* 1990; **57**: 783–95.
4 Katsarava R, Beridze V, Arabuli N *et al.* Amino acid-based bioanalogous polymers. Synthesis, and study of regular poly (ester amide) s based on bis (alpha-amino acid) alpha-alkylene diesters, and aliphatic dicarboxylic acids. *J Polymer Sci* 1999; **37**: 391–407.
5 Mi FL, Wu YB, Shyu SS *et al.* Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. *J Biomed Mater Res* 2002; **59**: 438–49.
6 Loke WK, Lau SK, Yong LL *et al.* Wound dressing with sustained anti-microbial capability. *J Biomed Mater Res* 2000; **53**: 8–17.
7 Markoishvili K, Tsitlanadze G, Katsarava R *et al.* A novel sustained-release matrix based on biodegradable poly (ester amide) s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. *Int J Dermatol* 2002; **41**: 453–8.
8 Shera G. Phage treatment of severe burns. *Br Med J* 1970; **1**: 568–9.
9 Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. *Antimicrob Agents Chemother* 2001; **45**: 649–59.
10 Saito T, Takeuchi R, Hirakawa K *et al.* Slow-releasing potential of vancomycin-loaded porous hydroxyapatite blocks implanted into MRSA osteomyelitis. *J Biomed Mater Res* 2002; **63**: 245–51.
11 Soothill JS. Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa*. *Burns* 1994; **20**: 209–11.
12 Stone R. Bacteriophage therapy. Stalin's forgotten cure. *Science* 2002; **298**: 728–31.

