Prolonged Bactericidal Effect of Sodium Hypochlorite

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Polyvinyl pyrrolidone prolongs the bactericidal effect of sodium hypochlorite (NaOCl) by 25-30 times. The antibacterial activity of immobilized NaOCl depends on the polymer concentration in the solution. Excess of polyvinyl pyrrolidone leads to blockade of active groups and reduces NaOCl activity.

Key Words: *polyvinyl pyrrolidone; staphylococcus; Escherichia coli; sodium hypochlorite*

High incidence of nosocomial infections and multidrug resistant strains of microorganisms necessitate search for new drugs and methods for controlling purulent surgical infection. The method of indirect electrochemical oxidation with sodium hypochlorite (NaOCl) is a perspective treatment. This agent is highly effective towards purulent surgical infection; it cancels antibiotic resistance of microorganisms; the effects of the majority of antibacterial drugs are potentiated by NaOCl; the exo- and endotoxins of pathogenic microorganisms can be bound by sodium hypochlorite [1,2, 4]. However, NaOCl is an unstable compound, and its therapeutic effect is limited by several minutes, after which it rapidly degrades. This limits the use of NaOCl in local therapy of purulent wounds.

We investigated the possibility of prolonging the bactericidal effect of NaOCl by immobilization on low-molecular-weight dextran.

MATERIALS AND METHODS

Sterile apyrogenic solutions of sodium hypochlorite were prepared by electrolysis of 0.89% NaCl in an automated mode on an EDO-4 device (Regnatis, Moscow). The concentration of NaOCl (600 and 1200 mg/liter) was regulated by changing the exposure and current during electrolysis. The agent was mixed (1:1) with sterile water solution of medical polyvinyl pyrrolidone (PVP) with molecular weight of 12600±2000 in different concentrations at 18-20°C. Under these conditions competitive interactions of weakly oriented anion groups led to fixation of active oxygen on the polymer [3].

Antibacterial activity of immobilized NaOCl was investigated using standard *Escherichia coli* J53 met pro-ZZ lac+ and *Staphylococcus aureus* 209 strains.

Cultures of standard strains were grown in oblique meat-peptone agar (MPA) for 16 h, and a bacterial suspension (4×10^9 CFU in isotonic NaCl) was prepared. The antibacterial effect of NaOCl depends on the initial concentration of microorganisms: the higher the concentration of microorganisms, the higher the effective concentration of NaOCl. For simulating the conditions in a purulent wound, 5 ml NaOCl with and without PVP was mixed with 0.2 ml fresh heparinized human plasma.

The mixture was incubated at 37°C for 24 h and 0.5-ml aliquots taken after certain intervals (15 min, 1, 6, 12, and 24 h) were incubated with 0.1 ml bacterial suspension for 15 min at 37°C in a water bath. The number of viable bacteria was evaluated by the method of serial dilutions after inoculation of the mixture in dishes with MPA (for *S. aureus*) or Endo medium (for *E. coli*). Various concentrations of NaOCl (600 and 1200 mg/liter) and PVP (0.5, 1, 2, 5, and 10%) were combined.

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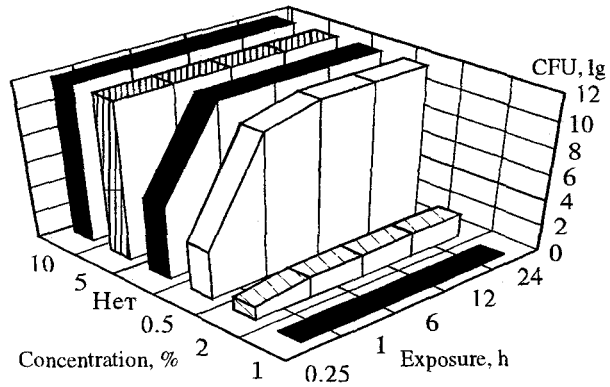

Fig. 1. CFU in a *E. coli* culture treated with NaOCl (600 mg/liter) and polyvinyl pyrrolidone.

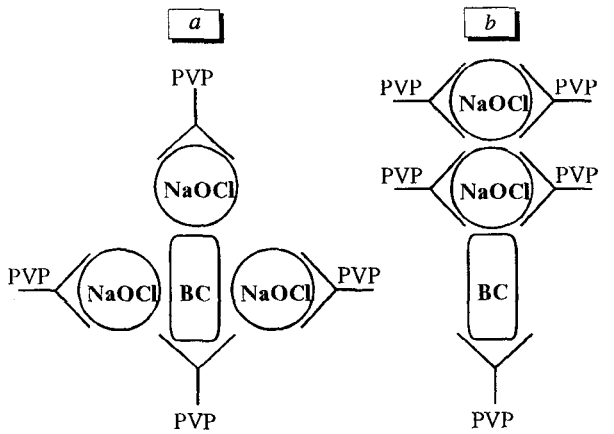

Fig. 2. Bactericidal effect of NaOCl at polyvinyl pyrrolidone (PVP) concentrations 1-2% (*a*) and >5% (*b*). BC: bacterial cell.

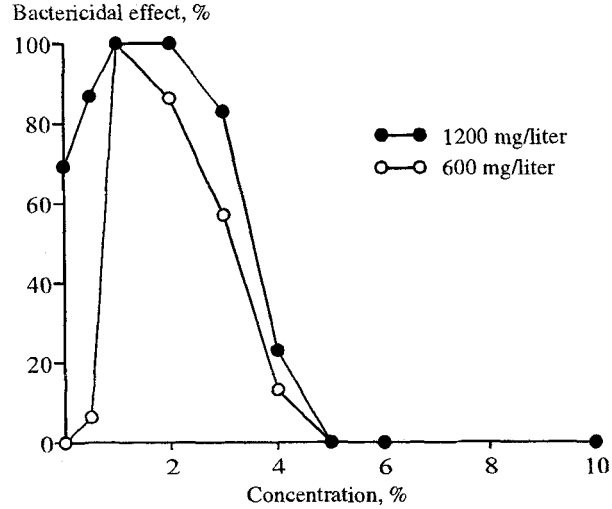

Fig. 3. Bactericidal effect of immobilized NaOCl for *E. coli* culture at different concentrations of polyvinyl pyrrolidone. Exposure 60 min.

In the control, NaOCl was replaced with 0.89% NaCl. In order to rule out the nonspecific effects of PVP on bacterial cells (for example, coagglutination), the numbers of CFU in bacterial suspension in isotonic NaCl and PVP were compared.

RESULTS

When standard *E. coli* J53 met pro-ZZ lac+ strain was incubated with NaOCl without PVP the antibacterial activity of the solution disappeared within 1 h (Fig. 1). Addition of PVP prolonged the antibacterial activity of NaOCl in a dose-dependent manner. In a concentration of 0.5% PVP prolonged the agent activity to 6 h. The maximum effect was observed at a PVP concentration of 1%, when the drug activity decreased only after 24 h. Further increase in PVP concentration in the mixture reduced its antibacterial activity. Mixtures containing more than 5% PVP possessed no antibacterial activity.

Similar results were observed with *S. aureus* cultures, though the antibacterial activity of immobilized NaOCl was lower by an order of magnitude. In the control series, isotonic NaCl did not suppress the viability of bacteria. Control tests with PVP in different concentrations without NaOCl showed that the number of CFU after exposure of bacterial suspensions for 15 and 30 min did not depend on the concentration of PVP and was virtually the same as in isotonic NaCl.

Hence, the bactericidal effect of NaOCl depends on the concentration of PVP in the solution. The effect of 1-2% PVP can be due to binding of bacteria and toxins to the carrier enabling their close contact with the oxidant (Fig. 2, *a*). The bactericidal effect in this case is due to immediate effect of free NaOCl in the solution and prolonged effect of a relatively unstable complex of PVP and hypochlorite dissociation products (hypochlorous acid HOCl and hypochlorite ion OCl^-). Supersaturation of the solution with PVP increases cross-linking of active polymer groups with NaOCl dissociation products and yields a tight matrix with blocked active groups. Therefore, bacterial cells contact only with surface active groups in this complex (Fig. 2, *b*), which was confirmed in experiments with different concentrations of NaOCl (Fig. 3).

Thus, polymeric carrier considerably (25-30 times) prolonged the bactericidal effect of NaOCl, while an excess of the polymeric carrier in the solution blocked active groups and sharply decreased antibacterial activity of the complex. Polymer-immobilized NaOCl is a promising agent for medicine: prolongation of its effect for more than 24 h makes it a useful drug for the treatment of intoxications and in pyoseptic surgery, particularly in infections caused by anaerobic microflora.

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