Evaluation of in vitro efficacy of the disinfectant Virkon

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Abstract. A study was conducted on a new acid peroxygen system based disinfectant (Virkon), in order to assess its in vitro efficacy. The chemical was tested on different bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli), spores (Bacillus subtilis) and on the Hepatitis B surface antigen (HBsAg), and compared in its activity with phenol and glutaraldehyde (calculation of the 'phenol coefficient' and the 'glutaraldehyde coefficient'). The constancy of speed of disinfection, the coefficient of concentration, the minimal inhibitory concentration (MIC) were also determined, and the destruction of the HBsAg antigenic activity was studied using an ELISA kit. The sporicidal efficacy of Virkon was assessed by cultivating spores in agar nutrient after contact with different dilutions of the disinfectant. The results of the tests showed that Virkon has a high concentration coefficient (mean value of k: 0.374/min) and a wide range of action. The low MIC demonstrates how little concentrations of Virkon can inactivate all studied bacteria. The disinfectant was also able to destroy the hepatitis B surface antigen, and it demonstrated good activity against spores, especially if used in physiologic solution. These characteristics, coupled with the absence of irritation or toxic effects on animals showed by other studies, make wide fields of application for the new disinfectant foreseeable.

Key words: Bacteria, HBsAg, Virkon

Introduction

The prevention of hospital acquired infection (HAI), the control of AIDS, the transmission of hepatitis during surgical or invasive treatments are crucial problems in public health.

In a general strategy for their solution, the correct use of the disinfectants is one of the means which can be usefully applied.

At present the chemicals used for this aim contain glutaraldehyde in different formulations and, frequently, in association with other active principles such as: phenol, sodium hypochloride, iodophor compounds, isopropyl alcohol, quaternary ammonium chemicals, etc.

Recently, a new type of disinfectant based on the acid peroxygen system (Virkon), was widely studied regarding its antimicrobial activity towards:
- several strains of different bacteria (Streptococcus faecalis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Salmonella enteritidis [1, 2], Mycobacterium tuberculosis, Mycobacterium avium complex [3, 4], Streptococcus pyogenes, Clostridium perfringens, Bacillus subtilis, Klebsiella oxytoca, Proteus vulgaris, Shigella sonnei, Neisseria gonorrhoeae, Neisseria meningitidis, Klebsiella pneumoniae [5]);
- different types of viruses (e.g. Hepadnaviridae, Retroviridae [6, 7]);
- some spores (Bacillus subtilis, Bacillus cereus, Clostridium sporogenes, etc. [8]);
- fungi and miceti (Candida albicans, Fusarium solani, Microsporum canis, Trichophyton verrucosum, Trichophyton mentagrophytes [1, 9, 10]).

The studies, performed in vitro under 'clean' and 'dirty' conditions and in operative circumstances (bronchoscopy endoscopy, etc.), showed very good antimicrobial activity [1, 3, 9, 11].

Other researches [12–14] pointed out the very low acute and chronic toxicity of Virkon when tested on rats.

Moreover, the disinfectant had no irritant effects on the bare skin [15] or the eyes [16] when tested on rabbits.

In order to prove the efficacy of this disinfectant its activity was studied in vitro under 'clean' condition, towards several strains of bacteria (also in comparison with phenol and glutaraldehyde), against the hepatitis B virus and versus spores of Bacillus subtilis. The results of the study are reported in this paper.
Materials and methods

**Virkon.** The disinfectant powder was provided by General Medical Supply (Grottaferrata, Rome, Italy). The disinfectant solution was made freshly, in sterile distilled water or in physiologic solution, at different concentrations: from 0.1 to 2%, but, usually, at 1% (w/v).

**Neutralising solution.** A solution (0.5% w/v) of sodium thiosulphate was applied for a period of 20 minutes.

**Test organisms.** *Staphylococcus aureus* ATCC 13150, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus subtilis* pb 168, hepatitis B surface antigen (HBsAg) from the positive sera tested by ELISA (ELISA, HBsAg, Sclavo Diagnostics) were used.

**Phenol.** Phenol (Fluka), in loose crystals, was utilised. The solution was prepared freshly, before every test, under a fume hood, with absolute filters (Securitas 2, Pool Bioanalysis Italiana).

**Glutaraldehyde.** Diba (Glaxo), a commercial product, was used. It contains the active principle at a concentration of 2%.

**Media.** Nutrient broth (Difco), nutrient agar (Difco), nutrient agar (Difco) added with manganese-chloride (2 ml of a solution at 1% every 100 ml of medium), were utilised.

**Determination of the constant of speed of disinfection.** k calculated from the results obtained by tests on *S. aureus*. We used subcultures incubated for 24 hours at 37 ± 1 °C. A freshly-made subculture was washed with physiologic solution and centrifuged at 1,500 r.p.m, for 10 min. The supernatant liquid was removed using a Pasteur pipette and the organisms resuspended in 10 ml of sterile physiologic solution. The number of organisms should not be less than 10⁴ per ml or more than 10⁷ per ml. Then, equal volumes of the disinfectant solution, at 0.1 and 0.2%, were added to dilutions (from 1/10 to 1/100,000), obtained from the suspension of the bacteria and left for 30 seconds and two minutes respectively. The disinfectant activity was stopped using sodium thiosulphate solution. Twenty minutes later a sample of 1 ml of the inoculum/disinfectant mixture was added to tubes of nutrient broth (3 for each dilution) and nutrient agar (2 plates for each dilution). All tubes and plates were incubated for 48 hours at 37 ± 1 °C.

In each test a positive control (organisms without Virkon) and a negative control (sterile physiologic solution instead of the organisms suspension) were included.

After 24 and 48 hours of incubation, the organisms were counted directly, with the aid of a light lens for the plates and by Most Probable Number (MPN) for the tubes.

The calculation of the k constant of speed of disinfection was made using the formula [17]:

\[
k = \frac{1}{t} \times \log \frac{N_0}{N_1}
\]

where: \( t \) = interval of time from \( t_0 \) to \( t_1 \);

\( N_0 \) = number of organisms at time \( t_0 \);

\( N_1 \) = number of organisms at time \( t_1 \).

**Determination of the coefficient of concentration.** n: The Virkon solution was used at 0.1 and 1% and the effect on *S. aureus* was studied after 15 and 40 seconds. The procedures, already reported for the k constant determination, were also used for the n coefficient determination. The n coefficient was calculated by the formula [17]:

\[
n = \frac{\log t_2 - \log t_1}{\log C_1 - \log C_2}
\]

where: \( t_1 \) and \( t_2 \) are the times needed for killing the same number of organisms using the 2 different concentrations (\( C_1 \) and \( C_2 \)) of Virkon.

**Method for determination of the minimal inhibitory concentration (MIC).** The MIC was measured, according to Finzi et al. [18], for the following types of bacteria: *S. aureus*, *P. aeruginosa*, and *E. coli*.

**Technique for the calculation of the phenol coefficient.** The *S. aureus* was tested. The coefficient was estimated according to Finzi et al.'s technique (partially modified) [18]. The modifications concerned mostly the dilutions. In fact 6 dilutions of phenol were made instead of 5; the 1/120 dilution was added, and Virkon was graded in a series of twofold dilutions from 1/8 to 1/1,024.

**Method for the comparison of Virkon with glutaraldehyde.** The technique performed was the same used for the phenol coefficient.

**Method for the evaluation of the damage on the hepatitis B virus after contact with the Virkon solution.** Five sera containing HBsAg were used. Two pools of sera were prepared, from 2 and 3 sera respectively. The pools were titred preliminary in order to find the best dilution to use. Several twofold dilutions were tested from 1/30 to 1/65,670 by means of an ELISA kit. The best dilution was 1/30. The dilutions of the two pools were subsequently added to the following mixtures:

- a) phosphate buffer saline solution and sodium thiosulphate 0.5% for a period of 20 minutes;
- b) Virkon and sodium thiosulphate;
- c) Virkon only and subsequent neutralisation after