The effect of super-oxidized water on Escherichia coli

V. Zinkevich, I. B. Beech, R. Tapper and I. Bogdarina

School of Pharmacy and Biomedical Sciences, University of Portsmouth, St. Michael’s Building, White Swan Road, Portsmouth PO1 2DT, UK

Summary: The mechanism of action of Sterilox, a non-toxic liquid biocide produced by electrolysis of a dilute saline solution, upon planktonic cells of Escherichia coli JM109 was investigated using protein and nucleic acid analysis. The results revealed total destruction of chromosomal and plasmid DNA, RNA and proteins of E. coli within 5 min of exposure. Our earlier investigation conducted using atomic force microscopy imaging revealed swelling and rupture of E. coli cells with release of cytoplasm. We propose that the biocidal properties of Sterilox are due to its effect upon constituents of the bacterial cell including proteins and nucleic acids.

Keywords: Super-oxidized water; Sterilox biocide; plasmid and chromosomal DNA damage; RNA damage; protein destruction; Escherichia coli.

Introduction

Sterilox is a liquid biocide made by electrolysis of a dilute saline solution within a proprietary electrochemical cell. It has been proposed as an alternative to glutaraldehyde for disinfection of flexible fibreoptic endoscopes and other heat-sensitive medical and surgical instruments. The exact chemistry of Sterilox, also referred to as super-oxidized water, has not yet been determined, however, the presence of HClO has been established. The redox value is typically >1000 mV and the pH varies from 2 to 14, but this may be controlled between 2.7 and 6.8. Although antimicrobial activity has been demonstrated for a variety of bacterial, fungal and viral species, the mechanism(s) by which it acts upon bacterial cells has not been reported.

In previous studies using atomic force microscopy (AFM) imaging we were able to establish that 5 min of exposure led to destruction of Escherichia coli cells which swelled and burst. The present investigation was undertaken to determine the effect of Sterilox on the cell constituents, such as nucleic acids (DNA and RNA) and proteins, of E. coli JM109.

Materials and methods

The Sterilox cell and production of biocide

The cell is composed of an outer titanium tube coated internally with rare earth oxides forming the anode, a ceramic diaphragm consisting primarily of alumina and a central titanium rod forming the cathode. Sleeves, caps, connectors and couplings are typically made from polypropylene or fluoroplast, and seals and gaskets in chemically-resistant rubber. A diaphragm separates the anolyte and catholyte solutions that are generated, respectively, at the anode and cathode of the cell. This prevents the two streams mixing and inter-reacting to form simple sodium hypochlorite. The biocide is produced by collecting the anolyte and catholyte solutions that are generated, respectively, at the anode and cathode of the cell. This prevents the two streams mixing and inter-reacting to form simple sodium hypochlorite. The biocide is produced by collecting the anolyte. The principal electrochemical reactions that take place at the electrodes
increase the redox potential to +1100 mV in the product stream (water has a redox potential of +200–+400 mV vs. a standard silver chloride electrode). The biocide was generated by pumping a solution of salt (5 M NaCl) in distilled water at a constant rate of 800 cm³/min through a prototype cell (Sterilox Medical Ltd.) via twin electrolysis units. The electrolytic decomposition of a dilute saline solution at a current of 9 A produced anolyte (biocide) with a pH of 5.5 and a redox potential > +1100 mV.

**Bacterial strains and growth conditions**

Plasmid-free cells of *E. coli* JM109 (Promega) and pUC19 (Stratagene) plasmid-carrying cells were used to assess the effect of Sterilox on proteins, DNA and RNA. Plasmid-free bacteria were grown as batch cultures in standard Luria Bertani (LB) liquid media. pUC19 plasmid-containing cells were grown in LB medium with 50 μg mL ampicillin (Sigma). After 6 h incubation at 37ºC, cells were pelleted at 20 300 g for 10 min at 4ºC and used for DNA, RNA purification and protein analysis.

**DNA and RNA preparation from normal and Sterilox-treated cells**

Genomic DNA was prepared from the bacterial cells using Genomic-tip 20/G (QIAGEN) according to the manufacturers’ instructions. Mini-scale plasmid preparations were carried out with the QIAGEN tip 20 plasmid purification kit (QIAGEN). Total RNA was prepared from *E. coli* JM109 collected from the log phase using RNAsy Mini Kit and QIAshredder for cell lysate homogenization (QIAGEN). Nucleic acids were analysed using a 1% agarose gel in standard tris-acetate buffer with ethidium bromide. DNA of pUC19 plasmid was obtained from New England Biolabs Inc.

**Treatment of cells and macromolecules**

Freshly prepared Sterilox was used for treating intact JM109 cells with and without plasmid pUC19 and the purified nucleic acids at a ratio of nine parts to one part of sample. The exposure time was 5 min at room temperature. To stop the action of the agent on bacterial cells, Tryptone (Merck) was added to give a final concentration of 10% and for nucleic acids albumin (Sigma) was added to give a final concentration of 5%. Controls consisted of macromolecules exposed to sterile saline solution.

**Polyacrylamide gel electrophoresis of protein**

Proteins were separated on a 10% SDS–polyacrylamide minigel system at a constant voltage of 150 V for 1 h. A low molecular weight protein standard was used (Bio-Rad) and the gel was stained with Coomassie blue R-250 (Bio-Rad) for 30 min at 100ºC.

**Results**

**Action on DNA and RNA**

The effect on the different types of DNA is presented in Figure 1(a). After 5 min exposure, chromosomal DNA of *E. coli* is undetectable (lane 2), whereas lane 1 clearly shows the presence of chromosomal DNA in the control. Lanes 3 and 4 show untreated and treated plasmid DNA pUC19, again revealing the absence of DNA after treatment. The effect on RNA is demonstrated in Figure 1(b).

**Figure 1** Analysis of DNA and RNA isolated from *E. coli* JM109. Purified DNA and RNA were either untreated or exposed for 5 min to Sterilox. (a) Lane 1, untreated chromosomal DNA; lane 2, chromosomal DNA after treatment with Sterilox; lane 3, untreated plasmid DNA pUC19; lane 4, Sterilox treated plasmid DNA pUC19. (b) Lane 1, total RNA of *E. coli* JM109 after treatment with Sterilox; lane 2, total RNA of *E. coli* JM109 without Sterilox treatment. The arrow indicates the position of RNA.
Sterilox destroyed all RNA (lane 1), whereas the control (lane 2) showed no damage.

**Action on treated bacterial cells**

Although the expected quantities of chromosomal DNA were recovered from the whole, untreated *E. coli* cells, [Figure 2(a), lane 1], the absence of DNA was apparent after exposure to Sterilox [Figure 2(a), lane 2]. The identical results were obtained with plasmid DNA from untreated cells [Figure 2(a), lane 3] and treated cells [Figure 2(a), lane 4]. The total RNA yields from the treated and control cells is shown in Figure 2(b), lanes 1 and 2 respectively. Only a trace of RNA is visible in lane 1.

**Action on proteins isolated from treated cells**

The activity upon the *E. coli* proteins is presented in Figure 3. Lanes 1, 3 and 6 show protein profiles from cells exposed to Sterilox. To generate a more visible image, different amounts of sample were used in each lane of the gel. Lane 1, which has the highest amount of material (10 μL) shows the disappearance of the protein bands, and only a general smear on the gel, whereas lane 2 (control) shows the normal protein profile of the *E. coli* cells. The quality of the profile reflects intentional overloading of the gel. Lanes 3 and 4 have half the amount of material (5 μL), in order to gain a better image on the gel. Lanes 6 and 7 have even less sample (2 μL), thus providing a clear image of protein separation.

**Discussion**

We have demonstrated that Sterilox acts upon *E. coli* JM109 cells by damaging double stranded DNA, RNA and proteins. Most probably, oxidizing chemicals in the compound destroy the covalent bonds in the nucleic acid chains, as well as in the protein chains. Our AFM study revealed that after 30s of exposure, cells of *E. coli* considerably increased in size. No intact cells were seen after 5 min of exposure, but a large amount of debris attributed to cytoplasmic material resulting from cell lysis was noted. Based on the AFM observations and data reported here, we propose that within 30s of exposure Sterilox was present inside the *E. coli* cell interfering with its metabolic activity, and causing structural and functional damage to cell membrane and/or to cell wall itself, as indicated by swelling. The final rupture of cell wall and leakage...
of cytoplasm within 5 min of treatment is due to the total destruction of proteins, DNA and RNA.

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References