Chemicals in disinfectants and sterilants

Discuss the uses and modes of action of chemicals used as disinfectants and sterilants

Disinfectants and sterilants have been available in a variety of forms for a great number of years. The pioneering work of Joseph Lister and Ignaz Semmelweis effectively opened the door to the range of chemicals that we have available today. Phenols (carbolic acid derivatives), mercuric chloride, chlorides, hypochlorites and iodine were the first major groups to be utilised. The quaternary ammonium compounds rose to prominence in the 1930s (Russell, A. D. 2002 [I]). The first detailed studies on the subject of mode of action were published by Cooper, who described the action of phenols as denaturing bacterial proteins as their main mode of operation (Cooper E A 1912)

Knaysi expanded this work further by suggesting that the order of death amongst cells was determined by some form of resistance (Knaysi G 1930) and that this might be linked to the protein structure of the cell wall (Knaysi G et al. 1930). Further work on phenol suggested that resistance of E. Coli was the same at virtually any concentration of phenol (Jordan, R. C et al. 1944) and this lead to the development of the concept of specific enzyme inhibition. (Roberts, M. H et al. 1946)

The term biocide is becoming more commonly used to describe the generic group. Russell (A D 2002 [II]) makes the comment that, until recently, two views permeated the field. One was that as long as they were effective, there seemed little merit in determining how they arrived at their inhibitory or lethal effects and secondly, that they were believed to act as protoplasmic

poisons and, as such did not merit much attention. We will present evidence to dispute both of these views.

Specific work on the mode of action of the biocides has been sporadic until comparatively recently. Gram-positive and -negative bacteria appear to have received the lion’s share of the research with less on the mycobacterium, less still on fungal and viral agents (Maillard, J.-Y et al. 1997), and few quality papers on the protozoa. (Turner, N. A et al. 1999). We know even less still on the ability of biocides to inactivate prions. (Taylor, D. M 1997).

Because of the wide range of potential biocidal (and biostatic) agents, it is not possible to even begin to tackle the question of mode of action in this essay on any but the most general terms

There are a number of current research issues such as the question of why it is that MIC’s of some cationic biocides such as chlorhexidine is similar for both mycobacterium and staphylococci yet they appear to possess low mycobacterium potency but are rapidly lethal to the staphylococci (Russell A D 1996)

Current considered opinion is that the majority (if not all) cells are not killed by a disactivation of a single target enzyme by the biocide. (Hugo, W. B. 1999)

Specific examples would be that enoyl reductase, (an enzyme involved in fatty acid synthesis) is totally inhibited by triclosan it is a combination of other intracellular disruptions that prove to be lethal to the cell. (Suller, M. T. E et al. 2000)

A number of researchers have commented on the apparent similarities between the disinfectants and sterilants group and the antibiotic group in terms of their effect on bacteria. To give specific examples, filament formation in Gm-ve. Bacteria is induced by both antibiotics (eg. ß-lactams andfluoroquinolones) and biocides (eg. phenoxyethanol and chloroacetamide) (Ng, E et al. 2002). Equally we have mentioned the action of triclosan on enoyl reductase, but this enzyme is also inhibited by isoniazid. (McMurry, L. M et al. 1999). A third category of similarity would be that the cell autolysis observed with both the phenols and some mercuric compounds is apparently the same as the processes observed with exposure to penicillin. (Hugo, W. B. 1999).

Other research groups (McKellar, R. C et al. 1996) have noted that exposure to some biocides can render a pathogen more susceptible to the action of antibiotics. It was postulated (although not proved) that this effect may be due to a structural change resulting in differing permeability in the cell wall of the microbe. (Morris, A et al. 1991)

It is not intended to imply that because these mechanisms appear similar that they are actually the biochemically or physiologically the same. Further research is needed to clarify these points

We have made comment on the apparent ability of some biocides to interfere with the integrity of the cell wall. In the case of the bacteria and fungi this may be a useful attribute to exploit, but it is a different matter in dealing with viral (and phage) vectors. The problem being that if the viral envelope – which is usually derived from the host cell – is damaged, then this

can result in the liberation of intact viral nucleic acid which, in itself, may be infective. (McClure, A. R et al. 1992)

If we consider one mechanism in some detail by way of an example, we could consider the action of chlorhexidine. This has been shown to produce a very rapid lethal effect (about 20 seconds) in both E. Coli and Staph. Aureus. (Denyer, S. P. 1995). It has been shown to produce damage to the outer cell membrane, but this action does not directly cause cell death. (El-Moug, T et al. 1985)

Chlorhexidine then crosses the damaged membrane by passive diffusion and causes leakage of the intracellular components. The cause of cell death is the inactivation of the intracellular constituents by protein denaturation of their controlling enzyme systems. This causes a further congealing of the cytoplasm and this reduces cellular leakage. This is the so-called biphasic mechanism of action of chlorhexidine. (Longworth, A. R. 1971).

There was a suggestion that chlorhexidine bound and inactivated the membrane-bound ATPase. It thereby disrupts the membrane potential and this is thought to be the prime mechanism of cell death .(Barett-Bee, K et al. 1994)

In conclusion, we have considered some of the mechanisms that are thought to be active in the bioactivity of disinfectants and sterilants. In doing so we should also consider the question of resistance. Many pathogens have efflux systems which can combat or eliminate biocides from their environment. These can work at comparatively low concentrations of the biocide. But it opens up the possibility of the development of biocide resistance in those

cells which survive the initial exposure (possibly due to incomplete treatment or peripheral exposure to low concentrations of agent). This mechanism selectively favours the emergence of biocide-resistant pathogens. (Azachi, M et al. 1996)

This issue has prompted calls for the rotation of disinfectants and sterilants in hospitals and elsewhere. (Benarde, M. A et al. 1967). The use of one disinfectant should ideally be replaced with another which has a completely dissimilar mode of action. This is one argument for the study of the mode of action of both disinfectants and sterilants.

Other arguments for studying the mechanisms of action of the biocides are many, not least is the fact that the knowledge of the mechanism of how a particular biocide can exert its effect can lead to the selective development of new targeted compounds with greater efficiency. An example of this process is the development of ortho-phthalaldehyde (OPA), which was specifically developed and adapted from “ older” compounds. (Behr, H et al. 1994)

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