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Modes of action of disinfectants

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Summary: *The exact mechanism of action of a disinfectant is not easy to elucidate. The notion of 'target' in the bacterial cell, frequently evoked for the antibiotics, is not clear for disinfectants (except for some, e.g. chlorhexidine). In understanding the mode of action of a disinfectant, it can be difficult to distinguish the primary stage (characteristic of the mode of action) and the secondary stage (consequence of the action). The author describes the actions of disinfectants on the external membrane, cytoplasmic membrane and energy metabolism of cells; these actions include rupture of the membrane, loss of permeability and coagulation of the cytoplasm.*

KEYWORDS: Bacteria – Disinfectant action – Disinfectants – Viruses.

INTRODUCTION

Disinfectants can act on microorganisms in two different ways: growth inhibition (bacteriostasis, fungistasis) or lethal action (bactericidal, fungicidal or virucidal effects). Only the lethal effects are of interest in disinfection and, as the objects of treatment have no inherent means of defence, lethality is the desired objective.

Although microbiologists have been working for more than a century on the problems associated with disinfection, understanding of the mode of action of active molecules remains vague: numerous hypotheses exist but few certainties. Many authors have long maintained that disinfectants and antiseptics act in a non-specific manner, in contrast to antibiotics which have distinct cellular targets within the microorganism. Although many studies still need to be performed in this field, it is clear that this distinction cannot be made for some molecules.

The discussion below focuses on the action of a certain number of active molecules. However, disinfectants are usually complex formulations of active molecules, sometimes also containing co-solvents, chelating agents, acidic or alkaline agents, or surface-active or anti-corrosive products.

It should also be noted that there may be considerable variation (in terms of pH, hardness, salinity, etc.) in the media surrounding the target microorganisms, and the state in which the latter is present (e.g. bacterium isolated or included in complex biofilm).

Understanding the mode of action of disinfectants requires an examination of the structure and functions of the bacterial cell.

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POSSIBLE STAGES OF THE MODE OF ACTION
(2, 4, 6, 9, 21)

In an analysis of the action of a disinfectant, it may often be difficult to distinguish between the primary stage (characteristic of the mode of action) and the secondary stage (merely a consequence of the action).

Action on the external membrane of the bacterial wall

A bacterium is protected from its environment by a membrane, the integrity of which is essential to survival of the bacterium. This membrane consists of basic compounds such as phospholipids and lipopolysaccharides, and is stabilised by Mg^{++} and Ca^{++} cations. Thus, if ionised disinfecting molecules are absorbed or repelled by electrical charges at the initial contact and absorption stage, the following means of action will theoretically be possible:

- non-polar molecules may dissolve and enter the lipid phase
- specific carrying systems will lead other molecules through the membrane
- other molecules will be able to disturb the organisation of the membrane by remaining bound to certain sites.

Action on the bacterial wall

The bacterial wall is important, as this confers rigidity and differs considerably between Gram-positive and Gram-negative bacteria. This diversity leads to great variation in the affinities of the hydrophilic disinfectants.

Action on the cytoplasmic membrane

An active molecule, such as a nutrient, may penetrate the cytoplasmic membrane in the following ways:

a) passive diffusion (non-specific and slow)

b) active transport (specific, enabling the accumulation of products in bacteria after either transformation or binding to a membrane protein).

Action on energy metabolism

Some disinfectants acting on adenosine triphosphatase (ATP) production are studied below (in the section 'Action of various disinfectants').

Action on the cytoplasm and nucleus

The disinfectant mechanism may operate on the cytoplasm and nucleus at the chromosome level.

Action on bacterial spores

The impermeability and the presence of dipicolinic acid in bacterial spores make these forms much more resistant to disinfectants than vegetative forms. The active disinfectants include highly oxidising products, such as hydrogen peroxide and chlorine, which can destabilise this structure in spores.

ACTION OF VARIOUS DISINFECTANTS

The following analysis is a review of the literature on a topic which has been continuously evolving since the 1950s. The author has ignored generalities, retaining only experimental data.

Acidic and alkaline compounds (6, 21)

The efficacy of acidic and alkaline agents is linked to the concentration of hydrogen (H^+) and hydroxyl (OH^-) ions, as follows:

– H^+ ions destroy the amino-acid bond in nucleic acids, modify the cytoplasmic pH and precipitate proteins;

– OH^- ions saponify the lipids in the enveloping membrane, leading to destruction of the superficial structure.

A pH higher than 10.0 disorganises the structure of the peptidoglycane and causes hydrolysis of the nucleotides of the virus genome. Similarly, the pH must exceed 12.0 to act on mycobacteria.

Chlorine and derivatives (1, 3, 6, 21, 22)

Chlorine is electronegative, and therefore oxidises peptide links and denatures proteins. Hypochlorite and chloramine in water produce hypochloric acid, which then decomposes. Both chlorine and oxygen are involved, and thiol groups are oxidised.

Exposure of strains of *Escherichia coli*, *Pseudomonas* spp. and *Staphylococcus* spp. to lethal doses of hypochloric acid causes a decrease in ATP production. Chlorine dioxide acts on the permeability of the external membrane of *E. coli* through a primary lethal phenomenon which consists in a substantial leakage of K^+ ions; such leakage does not occur for macromolecules. Sub-lethal doses inhibit cellular respiration due to a non-specific oxidising effect.

Quaternary ammonium compounds (5, 6, 17, 19, 20, 21, 23)

Quaternary ammonium compounds (QACs) irreversibly bind to the phospholipids and proteins of the membrane, thereby impairing permeability. The capacity of the bacterial cell to absorb such molecules influences sensitivity, as follows:

– The antimicrobial activity of quaternary ammonium with an alkyl chain is related to lipophilia and peaks between C_{12} and C_{16} (for both Gram-positive and Gram-negative bacterial strains).

– Several active compounds have less inhibitory effect on *Pseudomonas* spp. than on *Bacillus* spp., due to the presence of lipoproteins and liposaccharides on the outer layer of peptidoglycane.

– In *Pseudomonas* spp., the higher content of phospholipids and neutral lipids increases resistance. Benzalkonium chloride makes the cell more permeable. The same phenomenon is observed in *Enterobacter cloacae*.

– In Gram-positive bacteria, the product becomes bound to the wall proteins and is thus able to enter and destroy the membrane.

– Uniform absorption may be observed in Gram-positive and Gram-negative bacteria, corresponding to an increase in permeability and loss of viability

(e.g. cetyltrimethylammonium in *E. coli*). Electron microscopy reveals damage in *Pseudomonas aeruginosa* at the level of the outer membrane.

– In *Staphylococcus aureus*, cetyltrimethylammonium causes a leak in metabolites with low molecular weights (metabolic injury and modification of the permeability).

Amphoteric compounds (6, 14, 21)

A study of the action of dodecyldi(aminoethyl)glycine in two strains of *P. aeruginosa* shows that the amino-acid properties of this molecule enable it to enter the cell wall and the cytoplasmic membrane. The cell wall is thus punctured by tubular knobs.

Phenolic compounds (6, 10, 21)

Phenol acts specifically on the cell membrane and inactivates intracytoplasm enzymes by forming unstable complexes. The lipophilic molecules are trapped by the membrane phospholipids. The following processes are involved:

– If the concentration is low, the cell constituents (nucleic acids, glutamic acid) are liberated in the external media.

– If the concentration is high, the disinfectants inhibit permeases, thus causing denaturation of the bacterial proteins and lysis of the cell membrane.

In the case of *Bacillus megaterium*, for example, intracellular solutes are released from the testing cell or during growth (small solutes, such as protein derivatives), as a secondary effect of an interaction with enzymes bound to the cytoplasm membrane.

Peracetic acid and hydrogen peroxide (21)

Peracetic acid oxidises and denatures proteins and lipids of microorganisms, leading to disorganisation of the membrane. Swelling may take place in saturation of H^+ ions, which attract water. In many formulations, this action is difficult to dissociate from that of hydrogen peroxide.

Iodine compounds (6, 21)

Understanding the action of iodine-containing disinfectants requires study of the behaviour of iodine in aqueous or alcoholic solution.

Iodine-containing products are chiefly used for antisepsis of intact or damaged skin. However, iodine monochloride has recently been proposed as a disinfectant for inert surfaces.

Iodophors (complexes associating iodine with a solubilising or carrier agent) are also listed as iodine-containing products. The bactericidal activity of such products is determined by the galenic form.

Iodine acts by decreasing the oxygen requirements of aerobic microorganisms. Iodine interferes at the level of the respiratory chain of the microorganisms by blocking the transport of electrons through electrophilic reactions with the enzymes of the respiratory chain.

Iodine also interacts preferentially with the proteins of the cytoplasm membrane in a form with a positive ($H_2O + I$) or neutral (I_2 or HOI) charge.

Hydrogen peroxide (21)

Peroxides play an important part in degrading the bacterial cell.

In the presence of oxidising agents such as peroxides, bacteria such as *E. coli* defend themselves by producing enzymes which either destroy the oxidising agent before bacterial degradation takes place or help the restoring mechanisms.

Oxidised molecules are more sensitive to proteolysis than other molecules, and it has been suggested that a 'system of cell sanitisation' may enter into effect under the action of an oxidising agent such as H_2O_2.

Ozone (21)

Ozone probably acts on bacteria by oxidation. In viruses, ozone inactivates bacteriophages F2 and T4 by attacking the protein capsid to release and then inactivate the nucleic acids.

Aldehyde compounds (6, 21)

Formaldehyde

Formaldehyde acts on proteins by denaturation and on nucleic acids by alkylation, as follows:

$$O = CH_2 \Leftrightarrow \text{Protein} - NH - CH_2 - OH$$
$$\Rightarrow \text{Protein} - NH - CH_2 - NH - \text{Protein}.$$

The reaction is irreversible at the level of nucleic acids:

$$O = CH_2 \Rightarrow \text{Protein} - NH - CH_2 - NH - \text{DNA}.$$

The action of formaldehyde is identical at the ribo- and deoxyribonucleotide levels, except for guaniribo-deoxyribonucleotides. The 5′ dGMP (deoxy-guanosine monophosphate) interacts more rapidly with formaldehyde than 5′ GMP.

The reaction with receptive nucleotides occurs rapidly and the equilibrium shifts towards hydroxymethylation. This action is pH-dependent, working better at alkaline pH and less well at neutral or acid pH.

Glutaraldehyde (21)

The mode of action of glutaraldehyde is similar to that of formaldehyde. The action of glutaraldehyde is favoured by an alkaline pH (e.g. 8.0), but the solution is less stable in such conditions, the molecule is polymerised and the disinfectant activity decreases.

The addition of inorganic cations has been proposed, in combination with the effect of alkaline pH, to increase bactericidal activity.

The activity of glutaraldehyde is increased in the presence of magnesium ions (Mg^{++}), as more cellular alkaline phosphatase (PAse) is released. The PAse is found to be entirely localised in the periplasmic space at pH 6.8, whereas half of the PAse is released into the extracellular medium at pH 7.4. This may explain the observed difference in activity between acid and alkaline glutaraldehyde-based formulations. The presence of divalent cations at alkaline pH reinforces the bactericidal activity by concentration of the wall and/or plasmolysis of the bacterial cell. This potentiation of glutaraldehyde activity in the presence of Mg^{++} has also been reported in moulds and spores.

Biguanides

The mode of action of the polyhexamethylene biguanide (PHMB) family, which includes chlorhexidine, has been described in numerous publications (6, 13, 21).

The primary site of action is the cytoplasmic membrane, with resulting modification of membrane permeability. This effect was observed to be due to electrostatic interaction of the PHMB with the acid phospholipids in the cytoplasmic membrane. Given the size of the PHMB molecules, this implies actual absorption onto the cytoplasmic membrane of Gram-positive or Gram-negative bacteria, leading to a destructive effect at this level. Unlike certain antibiotics (penicillins, bacitracin and novobiocin), there is no accumulation of bacterial wall precursors; destruction is caused by rupture of the membrane and loss of permeability without lysis of the cell wall.

Release of the cell constituents occurs at very low concentrations. At the high concentrations used under antiseptic conditions, the bactericidal effect is very rapid, due to coagulation of the cytoplasm. Therefore, bacterial death is not due to leakage of the cell constituents at all concentrations of PHMB.

PARTICULAR MODE OF ACTION OF VIRUCIDAL DISINFECTANTS

The elucidation of the exact mechanism of action of a disinfectant against viruses is more difficult than for action against bacteria. Nevertheless, many studies on the susceptibility of viruses to chemical agents demonstrate that the following factors are important in understanding this action:

- presence of lipids in the viruses
- size of the viruses.

Noll and Youngner (18) classified the viruses in three groups:

- **group A**: lipid-containing viruses (e.g. Herpesviridae, Paramyxoviridae, Orthomyxoviridae)
- **group B**: small (20-30 nm), non-lipid viruses (e.g. Picornaviridae, Parvoviridae)
- **group C**: other non-lipid viruses (e.g. Adenoviridae, Reoviridae, Papovaviridae).

Over the last thirty years, many authors have reached the same conclusions – from very different *in vitro* tests – regarding the susceptibility of viruses to chemical agents: Klein and Deforest (11, 12), Scott (24), Derbyshire and Arkells (7), Evans *et al.* (8) and Maris (15, 16) all observe that the presence of lipid in a virus is uniformly associated with a high degree of susceptibility to all disinfectants; the absence of lipid and small size are associated with resistance to lipophilic chemical agents.

Fifty disinfectants with lipophilic properties (QACs, homologues of phenols, amphoterics, polymeric biguanides) are active against group A viruses and not against group B. However, chlorine and iodine compounds, oxidising agents, some aldehydes (glutaraldehyde) and strong acidic or alkaline agents are active against most viruses.

CONCLUSION

Although the notion of a target in the bacterial cell is frequently evoked in the case of antibiotics, this aspect remains rather vague with regard to disinfectants. Nor can disinfectant action be considered specific to a particular bacterial species, whereas such specificity is commonplace in antibiotics. At most, disinfectants may be said to have

bactericidal and/or fungicidal specificity. However, this obviously masks a certain disparity related to differences in activity between Gram-positive and Gram-negative bacteria.

One must also remember the principal parameters which condition the action and therefore the efficacy of a disinfectant (e.g. temperature, pH, concentration, time of contact).

The possible conjugated effect of several disinfectants also merits reflection. Again, as the modes of action are not always well defined, it is difficult to speak of a synergistic effect. At most, an additive effect is exerted when the concentrations of the active ingredients are suitably adapted. Due to the relatively scant knowledge of modes of action, and the existence of complex formulations, products from two different manufacturers should not be mixed, in view of the possible antagonistic effects of different products.

Brief mention must be made here of microbial resistance to disinfectants, which has no parallel with resistance to antibiotics. Manufacturers within the agricultural and food industries often claim that the bacteria on their premises have become resistant to the product used as disinfectant. Little is known of the microbial ecology of production units in these industries, as analysis in this field is not systematised. Nevertheless, various studies have shown that certain bacteria become resistant to chlorine following chlorination treatment of water. Future research should concentrate on discerning the mode of action of disinfectants on viable bacteria, bacteria under stress and bacterial components of a biofilm, under the normal conditions of use.

Under such conditions it will obviously be difficult to obtain a 99.999% reduction in the bacteria present, as required by the standards of the *Association française de normalisation* (French Standardisation Association).

Cleaning and disinfection must be fully effective, restricting contamination to an acceptable minimum level which is compatible with further manufacture.

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MODES D'ACTION DES DÉSINFECTANTS. – P. Maris.

Résumé : *Le mécanisme exact de l'action d'un désinfectant n'est pas facile à établir. La notion de « cible » dans la cellule bactérienne, souvent évoquée pour les antibiotiques, n'est pas claire pour les désinfectants, sauf pour quelques-uns d'entre eux comme la chlorhexidine. Dans l'analyse du mode d'action d'un désinfectant, la difficulté réside dans la distinction entre la phase primaire (caractéristique du mode d'action) et la phase secondaire (conséquence de ce mécanisme). L'auteur décrit les effets des désinfectants sur les membranes cellulaires externe et cytoplasmique ainsi que sur le métabolisme énergétique de la cellule. Il envisage en particulier la rupture de la membrane, la perte de perméabilité et la coagulation du cytoplasme.*

MOTS-CLÉS : Action désinfectante – Bactéries – Désinfectants – Virus.

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MODOS DE ACCIÓN DE LOS DESINFECTANTES. – P. Maris.

Resumen: *No es fácil determinar el mecanismo de acción exacto de un desinfectante. La noción de «blanco»* (target) *en la célula bacteriana, que suele usarse para los antibióticos, no es clara en el caso de los desinfectantes – salvo en el caso de algunos de ellos, como la clorhexidina. La dificultad para analizar el modo de acción de un desinfectante obedece a la distinción entre la fase primaria (característica del modo de acción) y la fase secundaria (consecuencia de ese mecanismo). El autor describe los efectos de los desinfectantes en las membranas celulares externa y citoplasmática así como en el metabolismo energético de la célula, y en particular la ruptura de la membrana, la pérdida de permeabilidad y la coagulación del citoplasma.*

PALABRAS CLAVE: Acción desinfectante – Bacterias – Desinfectantes – Virus.

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