Antibiotic Sensitivity of *Micrococcus radiodurans*

J. HAWIGER and J. JELJASZEWICZ

Department of Bacteriology, State Institute of Hygiene, Warsaw, Poland

Received for publication 27 September 1966

A wild-type strain of *Micrococcus radiodurans* and its nonpigmented mutant W₁ were tested for sensitivity to 10 antibiotics selected from the standpoint of their mechanism of action. Representatives of groups of antibiotics inhibiting deoxyribonucleic acid (DNA) synthesis, DNA-dependent ribonucleic acid synthesis, protein synthesis, and cell wall synthesis were selected. *M. radiodurans* and its mutant exhibited full susceptibility to all antibiotics tested (mitomycin C, actinomycin D, chloramphenicol, dihydrostreptomycin, erythromycin, neomycin, kanamycin, benzylpenicillin, bacitracin, and vancomycin), the degree of susceptibility being of the same order as that of a standard strain of *Staphylococcus aureus* 209 P, with the exception of dihydrostreptomycin.

*Micrococcus radiodurans* (1) is one of the microorganisms most resistant to γ radiation (21) and ultraviolet light (5). Besides its unusual radiation resistance, *M. radiodurans* differs from other cocci by fermentative properties and cell wall structure. The cell wall contains comparatively large amounts of lipids and a lipoprotein layer, as in gram-negative bacteria. The cell wall mucopolysaccharide contains L-ornithine, lysine, meso-diaminopimelic acid, and an unidentified amino acid (23).

Among colonies of *M. radiodurans* appearing after culture on medium that had previously been irradiated, two nonpigmented mutants, W₁ and W₂, were found. Their morphology, growth requirements, and high resistance to radiation are similar to those exhibited by the wild-type pigmented strain (11).

Experiments on the mechanism of radiation resistance suggest that a repair mechanism, probably of enzymatic nature and occurring during the logarithmic phase of growth, is involved (11, 19). This mechanism results in efficient repair of the deoxyribonucleic acid (DNA) molecule, and the cell continues its growth. Also of interest is the observation that, after re-exposure to ultraviolet, dimerization of thymine occurs in the DNA (19). A factor responsible for radiation resistance has been extracted from the cells of *M. radiodurans*; this factor in low doses sensitizes and in high doses protects *Escherichia coli* B/r from the action of γ radiation (4). Protective activity of *M. radiodurans* extracts has also been observed in mice subjected to γ radiation (J. P. P. Kilbourn, Thesis, Oregon State Univ., Corvallis, 1963).

The susceptibility of *M. radiodurans* to antibiotics is not known. The purpose of this paper was, therefore, the determination of sensitivity of the wild strain of *M. radiodurans* and its nonpigmented mutant to antibiotics. The following antibiotics were selected from the standpoint of their mechanism of action: influencing DNA synthesis, mitomycin C; DNA-dependent ribonucleic acid (RNA) synthesis, actinomycin D; protein synthesis, chloramphenicol, streptomycin, neomycin, kanamycin, and erythromycin; and cell wall synthesis, penicillin, bacitracin, and vancomycin.

**Materials and Methods**

**Strains.** A wild pigmented strain of *M. radiodurans* and its nonpigmented mutant, W₁, were received from B. E. B. Moseley of the Molteno Institute, University of Cambridge. *Staphylococcus aureus* 209 P was obtained from the Institute strain collection.

**Medium.** The medium used was recommended by B. E. B. Moseley (personal communication) and was composed as follows (grams per liter): pancreatic casein hydrolysate, 10.0; glucose, 1.0; yeast extract, 3.0; asparagine, 2.0; distilled water, 1,000 ml. The pH of the medium was 7.2. To obtain a solid medium, 15 g of Oxoid agar (Oxo Ltd., London, England) was added. Media were sterilized at 121°C for 20 min.

**Antibiotics.** The potencies and sources of standard antibiotics were as follows: mitomycin C, commercial sample from Kyowe Hakko Kogyo Co., Ltd., Tokyo, Japan; actinomycin D, laboratory standard received from Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.; chloramphenicol, reference standard, potency of 970 units per mg, from the Institute of Antibiotics (IA), Warsaw, Poland; dihydrostreptomycin, international standard prepared for the World Health Organization, potency of 760 IU/mg, from the Department of Biological Standards, National Institute for Medical Research (NIMR), London, England; erythromycin, inter-
Table 1. Antibiotic susceptibility of a wild strain of Micrococcus radiodurans, its mutant W₁, and Staphylococcus aureus 209 P

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>M. radiodurans W₁</th>
<th>M. radiodurans W₁</th>
<th>S. aureus 209 P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin C</td>
<td>0.78</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>0.39</td>
<td>0.78</td>
<td>0.19</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>3.12</td>
<td>3.12</td>
<td>3.12</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>0.04</td>
<td>0.09</td>
<td>0.78</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>Neomycin</td>
<td>0.19</td>
<td>0.19</td>
<td>0.39</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>0.39</td>
<td>0.78</td>
<td>0.39</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>0.39</td>
<td>0.39</td>
<td>0.78</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.39</td>
<td>0.39</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Antimicrobial standard prepared for the World Health Organization, potency of 950 IU/mg, NIMR; neomycin, reference standard, potency of 715 units per mg, from IA; kanamycin, international reference standard, potency of 1,000 units per mg, NIMR; penicillin G sodium salt, laboratory standard, potency of 1,530 IU/mg, Institute of Drugs, Warsaw; bacitracin, international reference standard prepared for the World Health Organization, potency of 55 IU/mg, NIMR; vancomycin, international reference standard prepared for the World Health Organization, potency of 1,000 units per mg, NIMR.

Determination of antibiotic susceptibility. The tube dilution method was used (9a). To prepare inocula, 18-hr cultures of M. radiodurans and S. aureus 209 P were diluted 1:1,000, and the mutant culture, 1:100. Strains were cultured on liquid medium containing antibiotics and were shaken continuously at 37°C. After 18 hr, the minimal inhibitory concentration (MIC) was recorded. Each experiment was repeated fourfold.

RESULTS AND DISCUSSION

The antibiotic sensitivity of the bacteria tested is presented in Table 1. With the exception of dihydrostreptomycin, there was no significant difference in susceptibility to the antibiotics of the strain of M. radiodurans, its mutant W₁, and S. aureus 209 P.

Mitomycin C was inhibitory for M. radiodurans at 0.78 μg/ml. The lethal activity of this antibiotic is caused by the formation of a heat-resistant cross-linkage between complementary strands of DNA (20). This suggests that M. radiodurans does not possess the repair mechanism or the ability to inactivate mitomycin which is present in liver or other tissue extracts (17, 18), and in Actinomyces mycelia (8, 20). These findings are of interest because, with Escherichia coli, cross-resistance to both mitomycin and ionizing radiation has been detected (2, 9).

Actinomycin D inhibited the growth of M. radiodurans at a concentration of 0.39 μg/ml. The susceptibility of this microorganism to actinomycin D is caused by formation of a complex with the messenger RNA (mRNA) synthesis-directing DNA that inhibits the DNA polymerase (7). For binding DNA with actinomycin, the presence of guanine in the DNA is necessary (10). Guanine is present in the DNA extracted from both the pigmented strain of M. radiodurans and the W₁ mutant (12).

Chloramphenicol acted on M. radiodurans and S. aureus at the same MIC level of 3.12 μg/ml. The influence of chloramphenicol on protein synthesis was first detected in staphylococci (6) and consists of the inhibition of activated amino acid transfer from tRNA to ribosomes (13), interference with amino acids polymerization (14), and probably also interference with incorporation of mRNA into ribosomes (15).

Erythromycin, inhibitory also for the three organisms tested at the same MIC level, acts similarly (22).

Dihydrostreptomycin was more inhibitory for M. radiodurans than for S. aureus 209 P. This antibiotic strongly inhibited the incorporation of phenylalanine within the acid-insoluble substances of ribosomes, and stimulated the incorporation of leucine and isoleucine, which disturbs the coding mechanism in the active protein synthesis center. Other cationic antibiotics, such as neomycin and kanamycin, have a similar mechanism of action (3). M. radiodurans is also susceptible to these antibiotics.

Penicillin, vancomycin, and bacitracin were equally inhibitory for the organisms tested. The action of these drugs is based on blocking of cell wall synthesis, which is manifested among other things by an accumulation of the uridine-muramyl peptides (16). Lack of difference in susceptibility of M. radiodurans and S. aureus 209 P is rather striking, as the mucopeptides of the cell walls of these microorganisms differ considerably. However, the pathways of mucopeptide synthesis may be similar in these two species, as suggested by the equal sensitivity to the action of antibiotics inhibiting this synthesis.

ACKNOWLEDGMENTS

We are indebted to B. E. B. Moseley of the Cambridge University for strains of M. radiodurans, and to all institutions for supplying the antibiotic standards used in this investigation.

LITERATURE CITED


