The Influence of Various Substances on the Gliding Motility of
Mycoplasma mobile 163K

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Non-toxic concentrations of various substances were tested for their influence on the gliding motility of Mycoplasma mobile 163K. A significant inhibitory effect on motility was observed with agents acting on nucleic acid synthesis (mitomycin), protein synthesis (puromycin, chloramphenicol), energy metabolism (p-chloromercuribenzoate, iodoacetate) and with compounds reacting with the cytoplasmic membrane or contractile elements (albumin, cholesterol, EDTA, 2-propanol, procain, CaCl₂, MgCl₂, colchicine and KI). The surface-active compounds Triton X-100, Tego and SDS increased the gliding velocity significantly in some concentrations and incubation periods. The results suggest that the motility of M. mobile depends on a functional cytoplasmic membrane and that cytoskeletal elements are involved in the gliding mechanism.

INTRODUCTION

Gliding motility has been described for five Mycoplasma species: M. pulmonis (Andrewes & Welch, 1946; Nelson, 1960; Nelson & Lyons, 1965; Bredt & Radestock, 1977), M. pneumoniae (Bredt, 1968, 1972; Bredt et al., 1970a, b; Radestock & Bredt, 1977), M. gallisepticum (Bredt, 1972, 1974; Erdmann, 1976), M. genitalium (Taylor-Robinson & Bredt, 1983) and M. mobile (Kirchhoff & Rosengarten, 1984; Kirchhoff et al., 1987). Motility studies on the first four species are difficult. These mycoplasmas glide optimally only at 37 °C and the movement is slow and interrupted by numerous resting periods. Therefore heated chambers and time-lapse photomicrographs are necessary to show the motility. This may explain why a comprehensive characterization of the movement has been performed only for M. pneumoniae (Radestock & Bredt, 1977). In contrast, M. mobile 163K glides optimally at room temperature and its movement is fast (2–4.5 μm s⁻¹, Kirchhoff et al., 1984) and not interrupted by resting periods, allowing direct microscopic observation of the movement. In the present paper the influence of several agents on the gliding movement of M. mobile is reported.

METHODS

Organism and growth. M. mobile 163K (Kirchhoff & Rosengarten, 1984; Kirchhoff et al., 1987) was grown in medium containing: Bacto PPLO broth, 16.8 g; bovine serum, 177.5 ml; freshly prepared yeast extract (50% w/v), 9.0 ml; penicillin/thallium acetate solution (2 × 10⁵ IU penicillin G ml⁻¹; 1.25%, w/v, thallium acetate), 9.0 ml; distilled water, 800 ml; pH 7.6. Solid medium was prepared by replacement of the Bacto PPLO broth by 28 g Bacto PPLO agar. All of the investigations were performed with mycoplasmas of the same batch. A stock culture (100 ml) of the tenth medium passage of M. mobile 163K was divided into samples of 2 ml, which were kept at −70 °C until use.

Reagents. The following substances were used: actinomycin (Fluka), mitomycin (Serva), puromycin (Serva), chloramphenicol (Sigma), p-chloromercuribenzoate (PCMB) (Fluka), iodoacetate (Serva), colchicine (Fluka), cytochalasin B (Serva), Triton X-100 (Serva), SDS (Serva), Tego 51 (Goldschmidt, Essen, FRG), 2-propanol

Abbreviation: PCMB, p-chloromercuribenzoate.
were prepared in microtitre plates using volumes of 10 μl per well and mixed with 190 μl medium containing 5 × 10^8 c.f.u. ml^{-1} (stock culture). Controls consisted of 190 μl mycoplasma stock culture and 10 μl sterile distilled water. The microtitre plates were incubated in a moist chamber at 25 °C. After 2, 4, 6 and 24 h two samples of 1-0 μl were taken from each well and spread on agar medium. The agar plates were incubated for 6 d at 25 °C and viability was determined.

Motility test. A frozen sample of M. mobile 163K was thawed and incubated for 20 h at 25 °C. Samples (190 μl) were transferred to wells of microtitre plates and mixed with 10 μl of various non-toxic concentrations of the substances to be tested. Controls received 10 μl distilled water. Plates were incubated in a moist chamber at 25 °C.

The influence of the substances on gliding motility was determined after 2, 4, 6-8 and 24 h incubation by comparing the speed of gliding of cells from the test and control wells. Samples (5 μl) were transferred to a slide, covered with a coverslip and examined by dark-field microscopy using a Leitz standard photo-microscope equipped with an oil-immersion dark-field condenser (numerical aperture 1.20) and an oil-immersion objective with an iris diaphragm (planachromat, 100 × , numerical aperture 1-10–1-30). For evaluation the ‘motility track’ technique (Vaituzis & Doetsch, 1969) was used. By this method the movements of the cells are recorded photomicrographically by long time exposures resulting in motility tracks on the film (see Fig. 1). Photomicrographs were taken with a Leica III g set-up camera on Kodak Tri-X Pan film (400 ASA) using an exposure time of 15 s. The negatives were framed and the slides were projected onto a screen on which the motility tracks were measured using a distance meter. The velocity of movement was calculated and the results examined for significance using Student’s t-test.

Morphological studies. Cellular morphology was assessed by electron microscopy of negatively stained preparations. Organisms were grown in Petri dishes on Pioioform films supported by electron microscope copper grids. The adherent cells on these films were directly stained with 2% (w/v) aqueous ammonium molybdate for 30 s without any preceding washing and fixation procedures. The grids were examined with a Zeiss model EM-10 electron microscope operating at 60 kV.

RESULTS

Table 1 shows the lethal concentrations of the substances and the concentrations which caused a significant reduction in c.f.u. For the investigation of their influence on motility only non-toxic concentrations of the compounds were used. The influence of these concentrations on the motility of M. mobile 163K is shown in Table 2 as the extent of deviation from the gliding speed of the control cells. A very strong inhibition of movement appears as a highly significant deviation (hs), a strong inhibition as a significant deviation (s), a weak inhibition as a weakly significant deviation (ws) and no inhibition as a non-significant deviation (ns). For some compounds (particularly mitomycin, cytochalasin B, albumin, PCMB, iodoacetate and colchicin) the effect varied with incubation time: maximum inhibition occurred in some cases only after 6–8 h incubation and, with most of these substances, the degree of inhibition decreased after 24 h.

A significant effect on motility was observed with substances acting on protein synthesis (puromycin, chloramphenicol) and energy metabolism (PCMB, iodoacetate), and with all of the substances reacting with the cytoplasmic membrane (Triton X-100, Tego, SDS, 2-propanol, MgCl₂, CaCl₂, EDTA, cholesterol, procaïn). Very high concentrations of MgCl₂ and CaCl₂ (about 1.58 M and 0.23 M, respectively) were required to decrease motility; these compounds inhibited the adherence of the mycoplasmas. This became clearly evident in the dark-field investigations: the organisms lost contact with the slide surface immediately after the addition of these substances. Physiological (about 100-fold lower) concentrations had no effect on adherence. Of the two compounds acting on nucleic acid synthesis (actinomycin and mitomycin) and the three affecting contractile elements (colchicin, KI and cytochalasin B), mitomycin, colchicin and KI (Fig. 1) inhibited gliding movement. The three surface-active compounds tested, Triton X-100, Tego and SDS, all caused a significant increase of the gliding velocity in some concentrations.

The electron microscopic investigation showed that the cells of M. mobile 163K treated with EDTA or KI lost their typical flask-shaped cell form (Fig. 2). This indicates that the cytoskeletal elements of the cells are probably destroyed by these substances.
Table 1. Lethal concentrations of various substances tested on M. mobile 163K

The concentrations shown are those which prevented growth. Concentrations which caused a significant reduction in the number of c.f.u. are given in parentheses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentrations (µg ml(^{-1})) toxic after an incubation time of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>Actinomycin</td>
<td>25-0 (12.5)</td>
</tr>
<tr>
<td>Mitomycin</td>
<td>6-25 (3-13)</td>
</tr>
<tr>
<td>Puromycin</td>
<td>&gt; 500 (&gt;500)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>&gt; 2500 (2500)</td>
</tr>
<tr>
<td>PCMB</td>
<td>125 (125)</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>&gt; 6250 (&gt;6250)</td>
</tr>
<tr>
<td>Colchicin</td>
<td>&gt; 250 (&gt;250)*</td>
</tr>
<tr>
<td>KI</td>
<td>1250 (313)</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>250 (5) x 10(^3)</td>
</tr>
<tr>
<td>Tego</td>
<td>5 x 10(^4)</td>
</tr>
</tbody>
</table>

* Shorter incubation times had the same mycoplasmacidal effect.

Table 2. Influence of non-toxic concentrations of various substances on the gliding motility of M. mobile 163K

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concn (µg ml(^{-1}))</th>
<th>Deviation from the speed of the control cells* after an incubation time of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>Actinomycin</td>
<td>0.78</td>
<td>ns (8/10)</td>
</tr>
<tr>
<td>Mitomycin</td>
<td>0.39</td>
<td>hs (24/22)</td>
</tr>
<tr>
<td>Puromycin</td>
<td>100</td>
<td>ns (12/10)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>125</td>
<td>ns (25/6)</td>
</tr>
<tr>
<td>PCMB</td>
<td>31</td>
<td>ns (26/16)</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>1.9-5</td>
<td>s (14/6)</td>
</tr>
<tr>
<td>Colchicin</td>
<td>6.25 x 10(^3)</td>
<td>hs (57/52)</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>250</td>
<td>s (18/36)</td>
</tr>
<tr>
<td>KI</td>
<td>5 x 10(^3)</td>
<td>hs (13/14)</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>3.2 x 10(^3)</td>
<td>s (11/15)</td>
</tr>
<tr>
<td>Tego</td>
<td>39</td>
<td>hs (17/16)</td>
</tr>
<tr>
<td>SDS</td>
<td>156</td>
<td>ns (28/52)</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>5 x 10(^3)</td>
<td>hs (11/15)</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>1.5 x 10(^3)</td>
<td>hs (5/12)</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>2.5 x 10(^4)</td>
<td>ns (12/25)</td>
</tr>
<tr>
<td>EDTA</td>
<td>5.5 x 10(^3)</td>
<td>hs (24/22)</td>
</tr>
<tr>
<td>Albumin</td>
<td>500</td>
<td>ns (17/13)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1 x 10(^3)</td>
<td>ws (7/15)</td>
</tr>
<tr>
<td>Procain</td>
<td>1.25 x 10(^3)</td>
<td>hs (32/16)</td>
</tr>
</tbody>
</table>

* ns, P ≥ 5% (not significant); ws, 1% ≤ P < 5% (weakly significant); s, 0.1% ≤ P < 1% (significant); hs, P < 0.1% (highly significant). Underlining indicates that the difference in speed was negative, i.e. that the gliding was faster than in the controls. The numbers in parentheses show the numbers of motility tracks evaluated: numerator, number of tracks from the test preparation; denominator, number of tracks from the control preparation.
DISCUSSION

There is no doubt that the cytoplasmic membrane plays an important role in the gliding movement of *M. mobile* 163K. All substances expected to act on the cytoplasmic membrane influenced movement. Concerning their action, a distinction can be made between substances which inhibit the adherence of the mycoplasmas (a precondition for the gliding movement) and substances which react with the membrane components responsible for gliding movement. An inhibition of adherence was caused by high (unphysiological) concentrations of Ca$^{2+}$ and Mg$^{2+}$ ions. Albumin also inhibited adherence, possibly by attaching to hydrophobic membrane components as shown by Feldner *et al.* (1983) for *M. pneumoniae*. Furthermore, EDTA and 2-propanol suppressed adherence, presumably by the deprivation of Ca$^{2+}$ and Mg$^{2+}$ ions from membrane proteins and by the denaturation of the proteins, respectively.
Gliding motility of Mycoplasma mobile 163K

Procain, cholesterol and colchicin had an influence on the membrane components involved in the gliding mechanism. It has been shown for eukaryotic cells (Singer & Nicolson, 1972; Furcht & Scott, 1975; Poste et al., 1975; Parks et al., 1979; Woda et al., 1980) that these substances cause an increase in membrane fluidity, induce changes in the arrangements of the membrane proteins, or disconnect the membrane proteins from the cytoskeletal elements. It is very likely that these substances act on the mycoplasma cell in the same manner, thereby inhibiting the gliding movement.

KI is generally known to be a 'chaotropic' substance, destroying highly polymerized cytoskeletal elements (Guba, 1950). This was also found by Göbel et al. (1981) in investigations on M. pneumoniae, in which KI depolymerized the actin-like filaments of this organism. KI probably acts in the same way on M. mobile. The movement of the cells was immediately inhibited after the addition of KI, suggesting a direct reaction with the components responsible for gliding. In addition, the cells lost their typical flask-shape and became rounded after treatment with KI. This can be interpreted as a destruction of the cytoskeleton.

Beside the inhibition of adherence, EDTA seems to affect cytoskeletal elements by increasing deprivation of Ca\(^{2+}\) and Mg\(^{2+}\) ions. Mg\(^{2+}\) ions are essential for the function of the cytoskeletal elements, as shown by Göbel et al. (1981), who found that the actin-like filaments of M. pneumoniae depolymerize in buffer lacking Mg\(^{2+}\) ions. In the present investigations, the movement of M. mobile 163K was inhibited promptly after the addition of EDTA. In addition, the cells lost their flask-shape. These are the same effects as those caused by KI, which destroys the cytoskeletal elements.

Cell components located on the surface of the cytoplasmic membrane are also involved in the gliding mechanism. This was indicated by the inhibition of the gliding movement by homologous antiserum (Rosengarten & Kirchhoff, 1987).

The mode of action of the surface-active compounds Triton X-100, Tego and SDS depended on their concentration and the treatment time. In some concentrations and incubation times they caused an increase in gliding speed, while in others they inhibited motility. The increase of the speed of movement was probably due to the reduction of the surface tension by these substances, provoking a reduction of the frictional forces. In higher concentrations and with longer incubation periods the surface-active compounds destroy the proteins in the cytoplasmic membrane (Kirchhoff, 1974) and obviously also those membrane proteins involved in gliding movement, thereby inhibiting motility.

Of the two substances interfering with DNA synthesis, mitomycin inhibited movement in some concentrations and incubation periods, suggesting that DNA synthesis may be necessary for the functioning of the motility mechanism, although actinomycin had no effect. The substances acting on protein synthesis had a delayed influence. Puromycin and chloramphenicol inhibited movement only after 6–8 h. This suggests that the proteins responsible for gliding movement have a relatively slow turnover. The inhibitory effects of PCMB and iodoacetate on movement show that cell-derived energy is necessary for gliding motion.

A satisfactory explanation for the variation of the results obtained with mitomycin, cytochalasin B, PCMB, iodoacetate, colchicin and albumin cannot be given. However, it may be related to the age of the cells, which strongly influences their sensitivity to certain compounds. This was observed in several experiments, especially in connexion with gliding motility.

In the present investigations, the motility of M. mobile 163K was influenced by several substances. Since we know at least partly the mode of action of these agents, limited conclusions can be drawn from the results obtained: an optimally operating cytoplasmic membrane is a precondition for gliding motility and a cytoskeleton may be involved in the gliding mechanism. Further studies are necessary to reveal more information about the gliding movement of mycoplasmas.

REFERENCES


