should therefore remind the pathologist to look for the presence of PPPN.

The cause of PPPN is still unknown. Genadry et al. regard it as a primary peritoneal tumour induced by a peritoneal irritant. Talc, asbestos or viruses are possible irritants, gaining entry into the peritoneal cavity via the genital canal. The ovaries, pelvic peritoneum and later the whole peritoneum are affected, but PPPN is an in situ lesion and can remain in this state for years.

The results of this study indicate that PPPN exists as a disease entity, and forms 6.5% of serous and papillary ovarian adenocarcinomas seen at Tygerberg Hospital. This 6.5% is of importance from the clinician's point of view, because of the very good prognosis. It is also important for research, not only in respect of a search for the causation of ovarian carcinoma but also in the evaluation of survival statistics. Inclusion of these patients in series of patients with ovarian carcinoma might falsely improve the survival rates.

REFERENCES


A comparison of the effects of tobramycin and netilmicin on the functions of human polymorphonuclear leucocytes and lymphocytes in vitro and in vivo

R. ANDERSON, A. C. FERNANDES, H. A. EFTYCHIS, G. JOONÉ, A. J. VAN RENSBURG

Summary

The effects of the antimicrobial agents tobramycin and netilmicin on the functions of human polymorphonuclear leucocytes (PMNLs) and on the mitogen-induced transformation of lymphocytes have been investigated both in vitro and in vivo before and 1 hour after a single intramuscular injection of the antibiotics. Neither antibiotic affected the migratory, phagocytic or antimicrobial capacities of PMNLs or the proliferative responses of lymphocytes to mitogens, at therapeutic concentrations or at 10-100-fold greater than therapeutic concentrations. Likewise, no alterations in these leucocyte functions accompanied the intramuscular injection of either antibiotic. Neither tobramycin nor netilmicin therefore interferes with host immunodefence mechanisms.


The aminoglycoside antibiotics gentamicin and amikacin at therapeutic concentrations have been reported to inhibit the migration of polymorphonuclear leucocytes (PMNLs) in vitro. Administration of these agents to normal healthy adults in therapeutic doses was also found to result in decreased PMNL chemotaxis. This decreased PMNL migration was transient, lasting about 24 hours, and followed the intramuscular injection of a single 500 mg dose of amikacin or an intramuscular injection of gentamicin at doses of 1,25 or 2,5 mg/kg. The authors concluded that their findings may be of clinical significance, especially when a patient with altered host defence mechanisms requires antimicrobial chemotherapy.
Although tobramycin and netilmicin have been reported to inhibit PMNL adherence and chemotaxis at therapeutic concentrations in vitro,1 less is known about the immunomodulatory effects of these antibiotics. We therefore investigated the effects of these agents on the functions of human PMNLs and on mitogen-induced transformation of lymphocytes.

Material and methods
Antibiotics
Tobramycin sulphate and netilmicin sulphate as pure substances and the injectable forms for human administration were obtained from Eli Lilly (Pty) Ltd, Johannesburg, and Scherag (Pty) Ltd, Johannesburg, respectively. The pure substances are freely water-soluble and their effects on PMNLs and lymphocyte functions were investigated over a concentration range of 10⁻³ M - 10⁻⁶ M, which corresponds to approximately 0.5 - 500 μg/ml. For in vivo studies prior informed consent was obtained from 6 healthy adult volunteers (5 females and 1 male) according to the requirements of the Ethics Committee of the Faculty of Medicine of the University of Pretoria. The volunteers were divided into two groups and blood specimens were taken for tests of PMNL and lymphocyte functions before and 1 hour after the intramuscular administration of a single dose of 1 mg/kg tobramycin or 1,7 mg/kg netilmicin. The dose of each antibiotic was calculated according to the manufacturers’ recommendations.

Bacteria
Reference strains of the test bacteria were kindly supplied by Professor H. J. Koornhof of the Department of Medical Microbiology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg. The strains used were Pseudomonas aeruginosa ATCC 10490, Escherichia coli ATCC 25922 and Klebsiella pneumoniae NCTC 11228. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for each strain are shown in Table I.

PMNL functions
Laboratory testing of PMNL migration using the leuko-attractant endotoxin-activated autologous serum (EAS) and of post-phagocytic oxidative metabolism (Candida albicans-stimulated luminol-enhanced chemiluminescence, hexose-monophosphate shunt activity and myeloperoxidase-mediated iodination) was carried out as previously described.4 PMNL phagocytosis of P. aeruginosa, E. coli and K. pneumoniae was measured by a radiometric technique. Bacteria were cultured overnight in nutrient broth containing 5 μCi radiolabelled amino acid mixture (¹⁴C-labelled L-lysine supplied by New England Nuclear, Boston, Mass., USA). After 18 hours’ incubation at 37°C the bacteria were washed three times with sterile Gey’s balanced salt solution (GBSS) and resuspended to 5 x 10⁶ colony-forming units per millilitre. The bacteria were pre-opsinized by incubating 0,3 ml of the bacterial suspension with 1,2 ml 5% fresh autologous serum. To measure phagocytosis, PMNLs and radiolabelled opsonized bacteria were incubated at a ratio of 1:10 in a reaction volume of 2 ml containing 5 x 10⁶ PMNLs and 5 x 10⁷ bacteria per millilitre. PMNLs were omitted from control systems. The tubes were incubated on a rotator at 37°C and 0,5 ml aliquots were transferred into 2,5 ml ice-cold GBSS after 5, 10 and 20 minutes’ incubation, centrifuged and washed twice to remove non-phagocytosed bacteria; the radioactivity associated with the PMNL pellet was measured in a liquid scintillation spectrophotometer. Results are expressed as the percentage of test bacteria phagocyted after correction for background values.

The effects of the antibiotics on phagocytosis were measured using two different systems: (i) the antibiotics were added to the PMNL/bacteria mixture before incubation and remained present throughout the 20-minute incubation period; and (ii) the test bacteria were grown overnight in sub-MICs of the antibiotics. The antibiotics were washed off after incubation and the antibiotic-exposed radiolabelled bacteria opsonized, and the rate of phagocytosis of these micro-organisms was compared with that of untreated control bacteria.

Mitogen-induced lymphocyte proliferation
This was measured as previously described using the mitogens phytohaemagglutinin (PHA) and concanavalin A (con A) at final concentrations of 2,5 and 5 μg/ml.4

Results
PMNL functions
Neither tobramycin nor netilmicin at concentrations up to 10⁻³ M (which is 100-fold greater than the therapeutic concen-

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**TABLE I. MICs AND MBCs OF TOBRAMYCIN AND NETILMYCIN FOR THE TEST BACTERIA (μg/ml)**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Tobramycin</th>
<th>Netilmicin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**TABLE II. EFFECTS OF INTRAMUSCULAR TOBRAMYCIN AND NETILMYCIN ON PMNL FUNCTIONS AND LYMPHOCYTE MITOGEN-INDUCED TRANSFORMATION**

<table>
<thead>
<tr>
<th></th>
<th>Migration to EAS (PMNLs/HPF)</th>
<th>Phagocytosis of C. albicans (%)</th>
<th>Phagocytosis-associated chemiluminescence (cpm)</th>
<th>Lymphocyte transformation (cpm) to PHA (2.5 μg/ml) Con A (2.5 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before tobramycin</td>
<td>173 ± 43</td>
<td>90 ± 2</td>
<td>93 599 ± 9 020</td>
<td>42 950 ± 3 700</td>
</tr>
<tr>
<td>1 h after injection of tobramycin</td>
<td>183 ± 2</td>
<td>93 ± 2</td>
<td>88 561 ± 19 197</td>
<td>37 750 ± 9 300</td>
</tr>
<tr>
<td>Before netilmicin</td>
<td>226 ± 14</td>
<td>95 ± 1</td>
<td>117 240 ± 23 970</td>
<td>36 380 ± 3 400</td>
</tr>
<tr>
<td>1 h after injection of netilmicin</td>
<td>226 ± 31</td>
<td>94 ± 1</td>
<td>116 179 ± 16 396</td>
<td>54 340 ± 10 006</td>
</tr>
</tbody>
</table>

*Results expressed as means ± SE for 3 subjects.
HPF = high-powered microscope field; cpm = counts per minute.
trations of both agents) affected PMNL migration to EAS, phagocytosis-associated chemiluminescence, hexose-monophosphate shunt activity or myeloperoxidase-mediated iodination. Likewise, phagocytosis of *Ps. aeruginosa*, *E. coli* and *Kl. pneumoniae* by PMNLs was not affected by either antibiotic (results not shown). Intramuscular administration of tobramycin or netilmicin to normal volunteers was not associated with any detectable alteration in PMNL migration to EAS, phagocytic activity or phagocytosis-associated chemiluminescence of PMNLs. These results are shown in Table II.

Effects of prolonged exposure of bacteria to sub-MICs of tobramycin and netilmicin on susceptibility to phagocytosis by PMNLs

In the case of *Ps. aeruginosa* bacterial growth was detected at concentrations of netilmicin of 0.1 μg/ml and 0.05 μg/ml and at a concentration of tobramycin of 0.05 μg/ml; in the case of *E. coli* and *Kl. pneumoniae* growth was detected at 0.005 μg/ml concentrations of both agents. However, exposure of bacteria to sub-MICs of the antibiotics did not significantly increase the susceptibility of treated bacteria to phagocytosis by PMNLs. Results for *Ps. aeruginosa* are shown in Fig. 1.

Lymphocyte transformation

Co-incubation of mononuclear leucocytes with either tobramycin or netilmicin at concentrations up to 10⁻³ M did not affect lymphocyte mitogen-induced proliferation. The control and 10⁻³ M tobramycin results were 32037 ± 1266 and 32981 ± 1284 radioactive counts per minute (cpm) respectively and the corresponding control and 10⁻³ M netilmicin results were 30812 ± 1241 and 28 956 ± 1 203 cpm. (Results are expressed as means ± SE of three different experiments using the mitogen PHA (2.5 μg/ml).) Intramuscular administration of tobramycin and netilmicin to normal volunteers was not associated with any significant change in mitogen-induced lymphocyte transformation (Table II).

Discussion

The results of this investigation have shown that neither tobramycin nor netilmicin inhibits the functions of human PMNLs and proliferative responses of lymphocytes to mitogens. It is therefore unlikely that either of these aminoglycoside antibiotics possesses immunosuppressive properties. No detectable effects of the antimicrobial agents on these leucocyte functions were observed at therapeutic concentrations or up to 100-fold greater than therapeutic concentrations in vitro or following the administration of the antibiotics to healthy adult volunteers.

REFERENCES