ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1985, p. 712–714
0066-4804/85/050712-03$02.00/0

Vol. 27, No. 5

Effects of Aminoglycoside Antibiotics on Polymorphonuclear Leukocyte Function In Vivo

FRANK R. VENEZIO* AND CATHY A. DiVINCENZO

Section of Infectious Diseases, Department of Medicine, Loyola University Stritch School of Medicine, Maywood, Illinois 60153

Received 28 January 1985/Accepted 26 February 1985

In vitro incubation of aminoglycoside antibiotics with human polymorphonuclear leukocytes (PMNs) has been shown to induce abnormalities in cell function. This study was designed to determine whether there are similar abnormalities in leukocyte function after exposure to the action of these agents in vivo. Four aminoglycosides (gentamicin, tobramycin, netilmicin, and amikacin) were tested. In vitro incubation did not induce a chemotactic defect when measured by an under-agarose method. However, inhibition of candidacidal activity was reproducible after in vitro incubation of all aminoglycosides tested. Nevertheless, when the aminoglycosides were administered intravenously to normal volunteers, PMN function, including adherence to nylon wool columns, chemotaxis, phagocytosis, and killing of *Candida albicans*, was unimpaired at 1, 3, and 24 h postinfusion. Therefore, we conclude that aminoglycoside antibiotic administration does not induce PMN dysfunction in vivo.

Aminoglycoside antibiotics have been reported by several investigators to induce suppression of polymorphonuclear leukocyte (PMN) functions, including inhibition of candidacidal activity (2), impairment of the chemotactic response (5, 15), and enhanced adherence as measured by nylon wool columns (15). All of these defects have been demonstrated in experiments in which various aminoglycosides were incubated with PMNs in vitro. Because the induction of abnormal neutrophil function by antibiotics could have deleterious clinical ramifications, especially in patients who have infections serious enough to require aminoglycoside therapy, this study was undertaken to determine whether functional PMN defects occur in vivo after the administration of commonly used aminoglycosides.

(These data were previously presented [F. R. Venezio and C. A. DiVincenzo, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 909, 1984].)

MATERIALS AND METHODS

Aminoglycoside antibiotics used. Four frequently used aminoglycosides were administered intravenously to normal volunteers between the ages of 23 and 37 years, and neutrophil function was measured at various intervals after infusion. The following aminoglycosides were administered in single 2-mg/kg doses: gentamicin (Garamycin; Schering Corp., Kenilworth, N.J.), tobramycin (Nebcin; Dista Products, Indianapolis, Ind.) and netilmicin (Netromicin; Schering Corp.). Amikacin (Amikin; Bristol Laboratories, Syracuse, N.Y.) was administered in a dose of 7.5 mg/kg. Each antibiotic was infused intravenously over 30 min in 5% glucose water.

Preparation of serum and neutrophil separation. Both whole and heparinized blood samples were drawn before administration of the aminoglycosides and 1, 3, and 24 h after the initiation of each infusion. Whole blood was allowed to clot and centrifuged to obtain serum. Purified PMNs were prepared from heparinized samples with Percoll gradients (6).

Adherence of PMNs in whole blood. Adherence of PMNs to nylon wool columns was measured by a method previously described (9). Briefly, 9-inch (ca. 23-cm) Pasteur pipettes were packed with 80 mg of nylon wool to a height of 15 mm. The PMNs were counted before 1 ml of blood sample was added to each of three columns. The remaining PMNs in the effluent were then enumerated. The adherence value was calculated with the mean of the three columns and was expressed as the mean percentage ± standard deviation.

Chemotaxis under agarose. PMN chemotaxis was measured by an under-agarose technique described by Nelson et al. (13). Briefly, purified PMNs were adjusted to a concentration of 2.5×10^7 cells per ml. A series of three wells was cut into agarose plates containing 0.75% agarose, 10% heat-inactivated serum (HIS), and minimal essential medium (MEM; GIBCO Laboratories, Grand Island, N.Y.). Zymosan-activated serum was placed in the outermost well, PMNs in the center well, and minimal essential medium in the inner well. After incubation, cell fronts were measured. Chemotaxis and random migration were quantitated by projection of the migration patterns with a microprojector (Tri-Simplex; Bausch & Lomb, Inc., Rochester, N.Y.). The chemotactic index (chemotaxis/random migration) and chemotactic differential (chemotaxis) − (random migration) were then calculated.

Phagocytosis and killing of *Candida albicans* after aminoglycoside infusion in vivo. A modification of the method of Lehrer and Cline was used to examine phagocytosis and killing of *C. albicans* (8). Purified PMNs obtained before and 1, 3, and 24 h after aminoglycoside administration were incubated with 10% autologous sera collected at these times.

TABLE 1. Aminoglycoside levels in serum[a]

Antibiotic	Mean level (μg/ml) ± SD in serum at following time after drug administration:		
	1 h	3 h	24 h
Gentamicin	8 ± 2	3 ± 1	<0.5
Tobramycin	11 ± 3	5 ± 1	<0.5
Netilmicin	10 ± 1	4 ± 1	<0.5
Amikacin	30 ± 3	15 ± 3	<0.5

[a] Each experiment was performed with five samples.

* Corresponding author.

TABLE 2. PMN adherence in volunteers after intravenous receipt of various aminoglycoside antibiotics[a]

Antibiotic	Mean % adherence ± SD at following time after drug administration:			
	0 h	1 h	3 h	24 h
Gentamicin	66 ± 2	67 ± 3	65 ± 4	66 ± 1
Tobramycin	67 ± 3	68 ± 3	66 ± 2	70 ± 4
Netilmicin	66 ± 1	66 ± 1	67 ± 1	65 ± 1
Amikacin	67 ± 2	66 ± 2	66 ± 3	70 ± 3

[a] Each experiment was performed in five volunteers.

The PMN-serum combinations containing various concentrations of aminoglycoside antibiotics were then incubated with *C. albicans* for 30 min, and smears were made to assess phagocytosis. The samples were incubated for an additional 30 min, and viability of the *Candida* isolates was determined by methylene blue exclusion. The phagocytic index was calculated by counting the number of ingested yeast cells per PMN on a Wright stained smear.

Phagocytosis and killing of *C. albicans* in vitro. Purified PMNs obtained from normal volunteers were incubated with various concentrations of aminoglycosides at 37°C for 30 min, and serum-opsonized *Candida* isolates were added. Phagocytosis and killing were performed as described above.

Drug levels in serum. The concentrations of the aminoglycosides in serum were determined by a microbiological agar diffusion technique previously described, with *Bacillus subtilis* as the test organism (14).

RESULTS

Concentrations of gentamicin, tobramycin, amikacin, and netilmicin in serum, within the therapeutic range, were achieved in all subjects 1 h after administration of these agents (Table 1). Concentrations 24 h postinfusion were less than 0.5 μg/ml in all subjects.

None of the aminoglycosides tested significantly altered neutrophil adherence from a mean baseline value of 67 ± 2% (Table 2). Composite adherence values for all the aminoglycosides were 67 ± 2% at 1 h after infusion, 66 ± 3% 3 h postinfusion, and 69 ± 4% at 24 h.

Chemotaxis of PMNs under agarose gel is shown in Table 3. Control PMNs migrated an average distance of 4.3 cm toward the chemoattractant. Chemotaxis was not significantly impaired in subjects receiving aminoglycosides at any time after dosage. Similarly, random migration of PMNs at all times tested did not deviate from the baseline value of 1.3 cm.

Because of the concern that Percoll treatment, washing, and resuspension of PMNs in media had removed all of the antibiotic and had led to falsely normal chemotaxis results, the following in vitro experiments were performed. PMNs from normal donors were suspended in HIS and HIS containing gentamicin, tobramycin, or netilmicin at 8 μg/ml or amikacin at 32 μg/ml. Chemotaxis was performed as described previously. Measurement of cell fronts revealed no impairment of chemotaxis or random migration with any aminoglycoside (Table 4).

TABLE 4. Chemotaxis of PMNs under agarose gel after in vitro aminoglycoside incubation[a]

Addition to PMNs	Chemotaxis (cm)	Random migration (cm)
HIS alone	4.2 ± 0.5	1.3 ± 0.2
HIS plus:		
Gentamicin (8 μg/ml)	4.3 ± 0.6	1.3 ± 0.1
Tobramycin (8 μg/ml)	4.6 ± 0.3	1.4 ± 0.1
Netilmicin (8 μg/ml)	4.4 ± 0.4	1.4 ± 0.1
Amikacin (32 μg/ml)	4.6 ± 0.2	1.4 ± 0.1

[a] Results are expressed as centimeters of PMN migration toward the chemoattractant (mean ± standard deviation). P = not significant for all values for HIS plus additions.

Progressive inhibition of candidacidal activity by in vitro incubation of normal PMNs with increasing concentrations of aminoglycosides is shown in Table 5. The candidacidal activities of neutrophils acquired from volunteers to whom the aminoglycosides had been administered are shown in Table 6.

The mean killing rate of 29% exhibited pretreatment remained essentially unchanged 1 and 3 h after intravenous aminoglycoside infusion. Phagocytosis of *C. albicans* by PMNs was demonstrated to be 3.8 yeast cells ingested per cell before aminoglycoside administration. This value also remained constant 1 and 3 h after infusion.

DISCUSSION

Ability of the host to recover from serious gram-negative bacillary infections is dependent not only on the antibacterial activity of various antibiotics but also on properly functioning PMNs. Many antimicrobial agents have been demonstrated to impair host immune activity, including delayed-type hypersensitivity (10), lymphocyte transformation (1, 3, 11), and various neutrophil functions (7, 15, 16). In particular, the effects of aminoglycosides on PMN function have caused much controversy. Several investigators have reported inhibition of chemotactic ability, as measured in Boyden chambers, after in vitro incubations of therapeutic concentrations of gentamicin (5, 15). On the other hand, no such inhibition was observed by Forsgren and Schmeling (4) with similar in vitro incubations measured by an agarose system. There is some minor disagreement as to the sensitivity of the methods used for the quantitation of chemo-

TABLE 3. Chemotaxis of PMNs under agarose gel after intravenous aminoglycoside infusion[a]

Antibiotic	Mean migration (cm) ± SD at following time after onset of infusion:		
	0 h	1 h	3 h
Gentamicin	4.4 ± 0.6	4.4 ± 0.6	4.1 ± 0.7
Tobramycin	4.3 ± 0.4	4.4 ± 0.4	4.9 ± 0.3
Netilmicin	4.2 ± 0.7	4.3 ± 0.6	4.2 ± 0.6
Amikacin	4.1 ± 0.7	4.2 ± 0.8	3.8 ± 0.6

[a] Each experiment was performed with five samples. P = not significant.

TABLE 5. Residual candidacidal activity of aminoglycoside-treated human PMNs

Antibiotic	% Activity at following drug level (μg/ml)[a]:				
	2	5	10	20	40
Gentamicin	98.0	76.4	64.7	52.9	43.1
Tobramycin	105	77.0	67.6	54.5	39.4
Amikacin	106	94.9	86.4	81.3	50.8
Netilmicin	90.4	82.8	76.0	68.0	54.4

[a] Performed as single experiments. Residual candidacidal activity = candida killed (+aminoglycosides)/candida killed (−aminoglycosides).

TABLE 6. Killing of candida by PMNs after intravenous aminoglycoside infusion[a]

Antibiotic	Mean % killed ± SD at following time after drug administration:		
	0 h	1 h	3 h
Gentimicin	27 ± 3	27 ± 4	27 ± 6
Tobramycin	32 ± 5	32 ± 5	32 ± 6
Netilmicin	29 ± 2	31 ± 4	31 ± 3
Amikacin	28 ± 3	28 ± 3	28 ± 3

[a] Each experiment was performed with five samples.

taxis. It has been suggested, however, that the under-agarose technique is as sensitive as the membrane filter assay in distinguishing defects in locomotion but that it may be less able to identify defects resulting from membrane deformability (12).

Because in vitro incubation of aminoglycosides was used in all of the experiments previously described, we undertook these experiments to correlate their results with data obtained after in vivo administration. In agreement with Forsgren and Schmeling (4), we could not demonstrate a defect in chemotaxis with in vitro incubation of gentamicin, tobramycin, netilmicin, or amikacin by a similar under-agarose method. However, we did reproduce the abnormality in candidacidal activity induced by in vitro incubation of increasing concentrations of these aminoglycosides, as previously demonstrated by Ferrari et al. (2). In contrast, we could find no impairment of neutrophil adherence, chemotaxis, phagocytosis, or killing after intravenous administration of gentamicin, tobramycin, netilmicin, or amikacin to normal volunteers in what would constitute a single loading dose under clinical conditions. Because these were healthy volunteers, ethical principles precluded the assessment of PMN function after multiple infusions of the various aminoglycosides, which would have more closely approximated their clinical application in serious bacterial infections. Because aminoglycoside levels were within the therapeutic range in all subjects 1 h after infusion, our results appear to have validity.

In summary, despite some reports demonstrating induction of various PMN defects after in vitro incubation with aminoglycoside antibiotics, we could show no PMN dysfunction after in vivo administration of any aminoglycoside tested.

ACKNOWLEDGMENTS

We thank the Eli Lilly Research Co. for their financial support.

We thank J. Lentino for consultation and D. Maslan for secretarial assistance.

LITERATURE CITED

1. **Chaperon, E. A., and W. E. Sanders, Jr.** 1978. Suppression of lymphocyte responses by cephalosporins. Infect. Immun. **19:** 378–384.
2. **Ferrari, F. A., A. Pagani, M. Marconi, R. Stefanoni, and A. G. Siccardi.** 1980. Inhibition of candidacidal activity of human neutrophil leukocytes by aminoglycoside antibiotics. Antimicrob. Agents Chemother. **17:**87–88.
3. **Forsgren, A., and G. Banck.** 1978. Influence of antibiotics on lymphocyte function in vitro. Infection **6**(Suppl. 1):91–97.
4. **Forsgren, A., and D. Schmeling.** 1977. Effect of antibiotics on chemotaxis of human leukocytes. Antimicrob. Agents Chemother. **11:**580–584.
5. **Goodhart, G. L.** 1977. Effect of aminoglycosides on the chemotactic response of human polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **12:**540–542.
6. **Hjorth, R., A. Jonsson, and J. Vielblad.** 1981. A rapid method for purification of human granulocytes using Percoll. A comparison with dextran sedimentation. J. Immunol. Methods **43:** 95–101.
7. **Lehrer, R. I.** 1973. Effects of colchicine and chloramphenicol on the oxidative metabolism and phagocytic activity of human neutrophils. J. Infect. Dis. **127:**40–48.
8. **Lehrer, R. I., and M. J. Cline.** 1969. Interaction of *Candida albicans* with human leukocytes and serum. J. Bacteriol. **98:** 996–1004.
9. **MacGregor, R. R., P. J. Spagnuolo, and A. L. Lentnek.** 1974. Inhibition of granulocyte adherence by ethanol, prednisone, and aspirin measured with an assay system. N. Engl. J. Med. **291:** 642–645.
10. **Mukerjee, P., S. Schuldt, and J. E. Kasik.** 1973. Effect of rifampin on cutaneous hypersensitivity to purified protein derivative in humans. Antimicrob. Agents Chemother. **4:**607–611.
11. **Munster, A. M., C. B. Loadholdt, A. G. Leary, and M. A. Barnes.** 1977. The effect of antibiotics on cell-mediated immunity. Surgery **81:**692–695.
12. **Nelson, R. D., R. T. McCormack, and V. D. Fiegel.** 1978. Chemotaxis of human leukocytes under agarose, p. 41. *In* J. I. Gallin and P. G. Quie (ed.), Leukocyte chemotaxis: methods, physiology, and clinical implications. Raven Press, New York.
13. **Nelson, R. D., P. G. Quie, and R. L. Simmons.** 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. **115:** 1650–1656.
14. **Sabath, L. D., and J. P. Anhalt.** 1980. Assay of antimicrobics, p. 485–490. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
15. **Seklecki, M. M., R. Quintiliani, and E. G. Maderazo.** 1978. Aminoglycoside antibiotics moderately impair granulocyte function. Antimicrob. Agents Chemother. **13:**552–554.
16. **Siegel, J. P., and J. S. Remington.** 1982. Effect of antimicrobial agents on the chemiluminescence of human polymorphonuclear leukocytes in response to phagocytosis. J. Antimicrob. Chemother. **10:**505–515.