Antimicrobial Agents and Chemotherapy

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***Antimicrob. Agents Chemother.* 1982, 21(6):1013. DOI: 10.1128/AAC.21.6.1013.**

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1982, p. 000–000
0066-4804/82/060001-00$02.00/0

Vol. 21, No. 6

Evaluation of Urinary Elimination of *N*-Acetyl-β-Glucosaminidase in Healthy Volunteers Treated with Dibekacin or Gentamicin

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Received 25 January 1982/Accepted 5 March 1982

The urinary excretion of *N*-acetyl-β-glucosaminidase was studied in healthy subjects during and after treatment with aminoglycosides. In terms of this parameter dibekacin appeared to be less nephrotoxic than gentamicin.

Dibekacin (3',4'-dideoxykanamycin B) is a recently introduced semisynthetic aminoglycoside antibiotic (3, 10, 11). The antimicrobial spectrum of activity of this drug appears to be similar to the spectra of gentamicin and tobramycin, but clinical effectiveness against gentamicin-resistant organisms has been reported (8). As with all aminoglycosides, dibekacin may be potentially nephrotoxic (5). Results obtained from comparative studies have been contradictory. Wold et al. (L. S. Wold, D. O. Robbins, C. L. Gries, B. L. Miller, and S. A. Turnipseed, Program Abstr. Annu. Meet. Soc. Toxicol. 1979, New Orleans, La., abstr. no. 39) evaluated the nephrotoxicity of dibekacin in rats by determining *N*-acetyl-β-D-glucosaminidase (NAG) levels and reported that this drug seemed to be more toxic than gentamicin. Working with the same species Rankin et al. (9) concluded that the toxicity of dibekacin was similar to, but not greater than, the toxicity of gentamicin after studying other parameters. This similarity was corroborated in healthy mongrel dogs by Whelton et al. (13), suggesting that dibekacin may be no more nephrotoxic than gentamicin. In a clinical study (N. Hirokawa, A. Haruyama, S. Oike, and T. Naruse, Abstr. 11th Int. Congr. Chemother., Boston, Mass., 1979) cancer patients were treated with a series of aminoglycoside antibiotics, and the amount of NAG excreted in urine was much higher in gentamicin-treated patients than in dibekacin-treated patients, with maximum NAG excretion occurring about 10 days after the initial dose. Another study (E. Puig, F. Dalet, R. Vila, and G. Del Rio, Abstr. 12th Int. Congr. Chemother., Florence, Italy, abstr. no. 1318, 1981) with patients with urinary tract infections indicated that gentamicin is more nephrotoxic than dibekacin and that nephrotoxicity (increase in NAG excretion) occurs more rapidly with gentamicin. These contradictory results with animal species and humans prompted us to study the potential nephrotoxicities of gentamicin and dibekacin in healthy human subjects. Two groups of male volunteers were treated with therapeutic doses of gentamicin or dibekacin for 10 days in a randomized, double-blind clinical trial.

A total of 16 healthy male volunteers 21 to 23 years old with normal renal function and without previous pathological antecedents of interest were selected. These volunteers were deprived of any medication for 30 days before the experiment. Informed consent was obtained from each volunteer, and the protocol of the assay was approved by the Research Committee of the Hospital de Nuestra Señora del Mar. The volunteers were randomly divided into two groups (eight volunteers each) and were treated simultaneously, either with gentamicin or with dibekacin at a dose of 3 mg/kg per day for 10 days; one-half of the total daily dose was administered intramuscularly every 12 h. The mean weight of the volunteers was 75.3 ± 6.8 kg (mean ± standard error of the mean). At the beginning and at the end of the trial, blood samples were analyzed for serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatases, urea, and creatinine, and urine samples were analyzed for proteinuria. The values for these parameters were within the normal ranges, and the treatment did not produce any alteration in them. We collected 24-h urine samples from each volunteer before treatment (days −3 and −1), during treatment (days 2, 4, 6, 8, 10), and after treatment (days +2, +4, and +6). The volume excreted was recorded, and 10-ml portions were stored at −20°C until they were analyzed for NAG excretion.

The excretion of hydrolases of renal origin in urine has been shown to be a useful index of various disorders of the kidney (4). Human urinary excretion of NAG is directly proportional to the corresponding rate of release by the

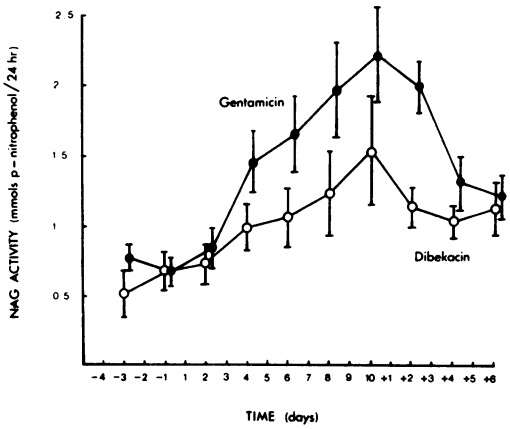

FIG. 1. NAG activity excreted in urine samples from healthy volunteers (eight volunteers in each group) after administration of gentamicin and dibekacin (3 mg/kg per day) for 10 days. The vertical bars indicate the standard error of the mean.

tubular epithelium (6) and has been used as an indicator of renal damage induced by renal disease (12) and potentially nephrotoxic drugs (2, 7). In this study the rate of NAG excretion was estimated by using 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide as the substrate, which offered significant advantages over other established procedures (6). The urinary NAG activity was determined by a modification of the methods of Borooah et al. (1) and Lockwood and Bosmann (6). Routinely, thawed urine samples (50 μl) were added to saline solutions (100 μl) containing 0.1% bovine serum albumin. A freshly prepared 0.05 M substrate solution in 0.05 M citrate buffer (pH 4.5) was added (850 μl) to the tubes, and the mixture was incubated for 1 h at 37°C in a metabolic shaker. The reaction was terminated by adding 2 ml of 0.25 M sodium carbonate, and the absorbance of the released *p*-nitrophenylate ion was determined spectrophotometrically at 420 nm. Each analysis was performed in duplicate, and parallel blanks of both urine and substrate were made. Activity was measured as millimoles of *p*-nitrophenol liberated in the 24-h urine samples by comparing the absorbance values of the samples with the values of solutions (10 to 200 μM) of *p*-nitrophenol in 0.25 M sodium carbonate. The reaction was routinely controlled by using a partially purified preparation of NAG from beef heart.

No significant differences in NAG excretion were observed in urine samples collected 1 or 3 days before treatment. The values obtained 1 day before the initial administration were taken as the basal levels (0.67 mmol of *p*-nitrophenol per 24 h for the two groups of volunteers). The administration of both gentamicin and dibekacin produced increases in NAG excretion from day 4 of treatment; NAG excretion reached a maximum on the last day of treatment (2.20 mmol of *p*-nitrophenol per 24 h for gentamicin treatment and 1.53 mmol of *p*-nitrophenol per 24 h for dibekacin treatment) and decreased thereafter (Fig. 1). Urinary NAG excretion after gentamicin treatment was significantly higher than the basal value from day 4 ($P < 0.01$), particularly at the end of the treatment period and on the following 2 days ($P < 0.001$). NAG excretion after dibekacin treatment differed significantly ($P < 0.05$) from the basal value only on day 2 after treatment. Nevertheless, NAG excretion on that day (1.13 mmol of *p*-nitrophenol per 24 h) was still significantly less ($P < 0.05$) than NAG excretion after gentamicin treatment (1.97 mmol of *p*-nitrophenol per 24 h).

The increases in the amounts of NAG in urine produced by the antibiotics were calculated as better indexes of the nephrotoxicities of the compounds. Thus, the actual increases in the amounts of NAG produced by the gentamicin and dibekacin treatments were calculated individually (subtraction of the basal NAG level in the absence of treatment from the total NAG excreted daily) and integrated with time by the trapezoidal rule. This calculation was made since the basal excretion of NAG fluctuates around a constant value, which is characteristic for each individual (12). The results of this experiment are presented in Table 1. Both aminoglycosides caused an increase in the amount of NAG compared with the basal levels, although the excretion induced by gentamicin (7.99 mmol of *p*-nitrophenol at the end of treatment) was twice that induced by dibekacin (3.55 mmol of *p*-nitrophenol). This trend was also maintained on the days after the last administration (Table 1).

Our results confirm that urinary excretion of NAG is of value for the study of the nephrotoxic potentials of aminoglycosides in humans. The previously reported increases in urinary NAG excretion in gentamicin-treated patients were detected in healthy volunteers. In the present study the urinary NAG excretion by gentamicin-treated volunteers was consistently higher than the urinary NAG excretion by dibekacin-treated subjects. In terms of NAG excretion, the difference between these two aminoglycosides was similar to the difference reported in subjects with urinary tract infections and lower than the difference reported in cancer patients. We concluded that in both patients and healthy subjects, dibekacin has less potential nephrotoxicity than gentamicin. More clinical trials in patients with distinct pathological conditions are still needed to establish the comparative safety of diverse aminoglycoside therapies since animal

TABLE 1. Cumulative increases in urinary NAG activity over basal levels during and after the administration of 3 mg of gentamicin and dibekacin per kg per day for 10 days to healthy volunteers

Time (days)	Urinary NAG activity in volunteers treated with:[a]		P
	Dibekacin	Gentamicin	
2	0.07 ± 0.03[b]	0.18 ± 0.09	NS[c]
4	0.46 ± 0.14	1.14 ± 0.26	<0.05
6	1.17 ± 0.31	2.90 ± 0.58	<0.05
8	2.13 ± 0.57	5.17 ± 0.93	<0.05
10	3.55 ± 0.96	7.99 ± 1.30	<0.05
+2	4.88 ± 1.23	10.83 ± 1.57	<0.05
+4	5.70 ± 1.34	12.76 ± 1.73	<0.01
+6	6.52 ± 1.43	13.93 ± 1.86	<0.01

[a] Expressed as millimoles of *p*-nitrophenol per 24-h urine sample.
[b] Mean ± standard error of the mean.
[c] NS, Not significant.

data seem not to be extrapolated easily to humans.

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