PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **189**, 338–343 (1988)

Adaptation to Nephrotoxic Chemicals (42816)[1]

MAUREEN B. JENNINGS AND MARCUS M. REIDENBERG[2]

Departments of Pharmacology and Medicine, Cornell University Medical College, New York, New York 10021

Abstract. Rats given gentamicin chronically become resistant to its nephrotoxic effects. To further explore this adaptation to nephrotoxicity, we gave male rats gentamicin 40 mg/kg/day for 12 days, then 80 mg/kg/day for 24 days. We then challenged them with 110 mg/kg/day of gentamicin for 9 days. Spermine was given 16 mg/kg/day for 42 days, then gentamicin challenge at 60 mg/kg/day for 9 days. Gossypol was given at 6 mg/kg/day for 19 days, then gentamicin at 60 mg/kg/day for 21 days. A fourth group of rats (controls) received 0.5 ml saline daily for 42 days and then received gentamicin 60 mg/kg/day for 9 days. Urine *N*-acetyl-β-glucosaminidase (NAG) was measured 3 times weekly and serum creatinine was measured 5 times during the study. Each drug-treated rat increased its urine NAG from baseline values. After a period of drug administration, all NAG values returned to the predrug values. Then all animals were given gentamicin daily. NAG values increased 20-fold in the animals previously treated with saline but did not rise in the other groups. The serum creatinine frequently but not always changed in parallel with the NAG values. These observations indicate that adaptation to these nephrotoxic substances occurs and that cross-resistance to gentamicin is produced by spermine and gossypol.

The resistance of the kidney that has recovered from chemically induced acute tubular necrosis to injury from a second dose of the nephrotoxic chemical was first observed by Suzuki in 1912 (1) and studied in pathologic detail by MacNider in 1929 (2). This acquired resistance to acute renal failure continues to be of interest and has been the subject of recent reviews (3, 4). Uranium salts were used for the early studies and $HgCl_2$ and gentamicin for the more recent ones. Studies evaluating cross protection have shown certain chemicals protect while others exacerbate the challenge. Glycerol and $HgCl_2$ cross protect. Dichromate and $HgCl_2$ protect against gentamicin challenge. Netilmicin exacerbates a gentamicin challenge and gentamicin exacerbates an $HgCl_2$ challenge (3).

The eventual recovery from the nephrotoxic effects of gentamicin during continued drug administration has been reported (5, 6) and studied in some detail (7, 8). The purpose of our research was to explore further the ability of the kidney to recover from nephrotoxic effects while continuing to receive the nephrotoxic drug and to examine further the extent of cross-resistance. Gentamicin was selected for the known positive control. Spermine was used because it is an endogenous metabolite that is known to be nephrotoxic (9, 10) and accumulates in the serum of some elderly people (11). Gossypol was selected because it is a natural product from cotton seed and was reported as causing an increase in urinary *N*-acetyl-β-glucosaminidase (NAG) excretion in some men receiving it in a clinical trial as an oral contraceptive (12) and caused a slight increase in serum creatinine in a chronic rat study (13) but not in a chronic primate study (14). Gossypol, when used as a contraceptive, is known to cause hypokalemia (14), a suspected nephrotoxic effect.

Renal tubular cells are rich in enzymes that may be released following damage. As reported by Prescott, the measurement of the activity of these enzymes in urine seems to be a sensitive test of proximal tubular injury by toxins (15). The increase in NAG may occur even in the absence of other evidence of renal injury and may represent simply a response

[1] Supported by a grant from the National Institutes of Health under Grant AM07661 and The Rockefeller Foundation.

[2] To whom reprint requests should be addressed at Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

0037-9727/88 $1.50

of the renal tubular cell to a drug or chemical. Whether evidence of injury or merely effect, the rate of NAG excretion can be used to investigate the renal response to drugs and chemicals.

Materials and Methods. Two experiments using the same protocol were conducted at different times. Male Sprague–Dawley rats weighing 200–300 g were housed singly in metabolic cages. Water and Purina diet were allowed *ad libitum.* The results of the two experiments were similar so they have been grouped together for analysis.

Gentamicin sulfate, spermine hydrochloride, and the 4-methylumbelliferyl derivative of *N*-acetyl-β-glucosaminide were purchased from Sigma Chemical Co., St. Louis, Missouri. Pharmaceutical grade gossypol acetic acid was supplied by the World Health Organization, Geneva, Switzerland.

Preliminary dose-ranging experiments revealed that a dose of gossypol of 6 mg/kg/day given sc was tolerated well by rats and was associated with normal weight gain after a few days of impaired weight gain. Spermine, 33 mg/kg, was reported as having systemic toxicity (10) so a dose of one-half that amount was chosen for these experiments.

Each experiment was started with four groups of four rats each. All drugs were given by subcutaneous injection 6 days per week. Group A received gossypol at 6 mg/kg/day for 19 days. Group B received spermine at 16 mg/kg/day for 42 days and Group C received gentamicin at 40 mg/kg/day for 12 days. Due to lack of urine NAG increase, the gentamicin dose for Group C was then increased to 80 mg/kg/day for 30 days. Group D received saline 0.5 ml/day. The transient rise in NAG excretion was seen within 2 days of gossypol administration while gentamicin required 21 days before an increase was detected. Spermine required 15 days for an initial increase in NAG. NAG had returned to baseline in the gossypol group after 19 days and in the spermine and gentamicin groups by 42 days of drug administration. Since we wanted to test for "adapted" kidneys, the gentamicin challenge was given after these NAG excretion levels returned to predrug values. Urine specimens were collected three times per week and *N*-acetyl-β-glucosamindase activity and creatinine concentration values were measured. After the return to predrug NAG values, a challenge dose of gentamicin 60 mg/kg/day was given to Groups A, B, and D. Group C (the gentamicin-treated group) was given a larger challenge dose of gentamicin 110 mg/kg/day. Because the results of the two experiments were similar, they were pooled for analysis.

Serum creatinine was measured at baseline, 2–3 days after the NAG peak, 2–3 days after the NAG returned to baseline, and during the subsequent gentamicin challenge. These days were chosen because serum creatinine elevation lags behind the proximal tubular necrosis and we tried to take blood at presumed times of high serum creatinine.

Urinary NAG was assayed by a modification (16) of the method of Leaback and Walker (17). The urinary NAG activity is expressed as nanomoles of 4-methylumbelliferone liberated from 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide substrate per hour of incubation at 37.0°C per milligram of urine creatinine.

Creatinine in serum and urine was measured by the alkaline picrate (Jaffe) reaction.

All statistics were done using the Mann–Whitney *U* test. All *P* values obtained were multiplied by four for the number of groups (three drug groups plus one saline (control group)) analyzed simultaneously (Bonferroni correction). In each analysis, the data for the selected day are compared to the predrug baseline value. This corrected *P* value is presented in the figures.

Results. Gentamicin, spermine, and gossypol produced an increase in *N*-acetyl-β-glucosaminidase excretion with an eventual return to predrug levels despite chronic drug administration. Detailed observations of the chronic gossypol experiment are shown in Fig. 1. The NAG levels rise within the first 2 days of gossypol administration and return to baseline by Day 6. A gentamicin challenge of 60 mg/k/day beginning on Day 19 failed to raise the NAG level during 21 days of gentamicin administration. Serum creatinine rose from a baseline value of 0.5 ± 0.1 to 0.9 ± 0.3 mg/dl after 3 days of gossypol administration. Figures 2 and 3 show the NAG excretion and serum creatinine course for chronic gentamicin and spermine injections, respectively, and the lack of effect of a

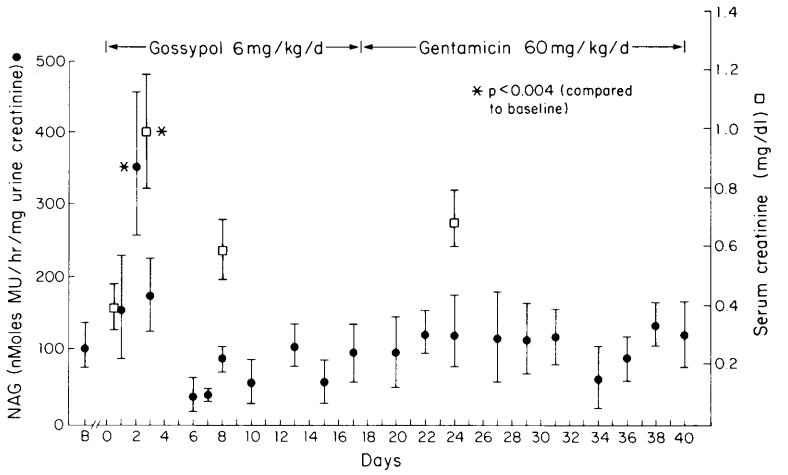

FIG. 1. Effect of 6 day per week gossypol (6 mg/kg) injections on urinary *N*-acetyl-β-glucosaminidase (NAG) and serum creatinine levels (mean ± SD). On Day 19, a gentamicin challenge at 60 mg/kg 6 days per week was begun. $N = 8$.

gentamicin challenge. The NAG levels rise within 22 days of chronic gentamicin administration and 16 days of chronic spermine injections with an eventual return to predrug levels for both nephrotoxins. For the spermine group, the serum creatinine doubled from a baseline value of 0.5 ± 0.1 to 1.0 ± 0.3 mg/dl after 15 days of treatment. The serum creatinine for the gentamicin group more than doubled from a value of 0.4 ± 0.1 at baseline to 1.0 ± 0.2 mg/dl after 21 days of treatment. Figure 4 shows the NAG course for chronic saline injections and the effect of a gentamicin challenge of 60 mg/k/day beginning on Day 42. The levels for the first 42 days in the saline group are presented to show the variability of NAG excretion values.

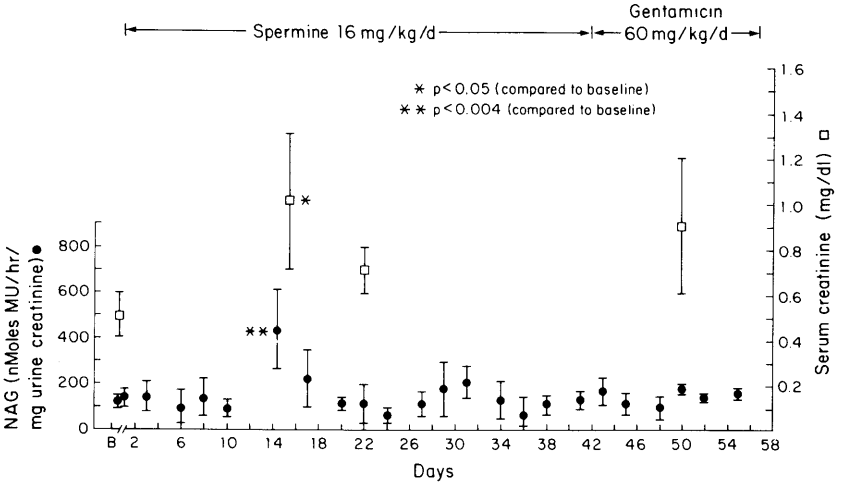

FIG. 2. Effect of 6 day per week spermine (16 mg/kg) injections on urinary *N*-acetyl-β-glucosaminidase (NAG) and serum creatinine levels (mean ± SD). On Day 42, a gentamicin challenge at 60 mg/kg 6 days per week was begun. $N = 6$.

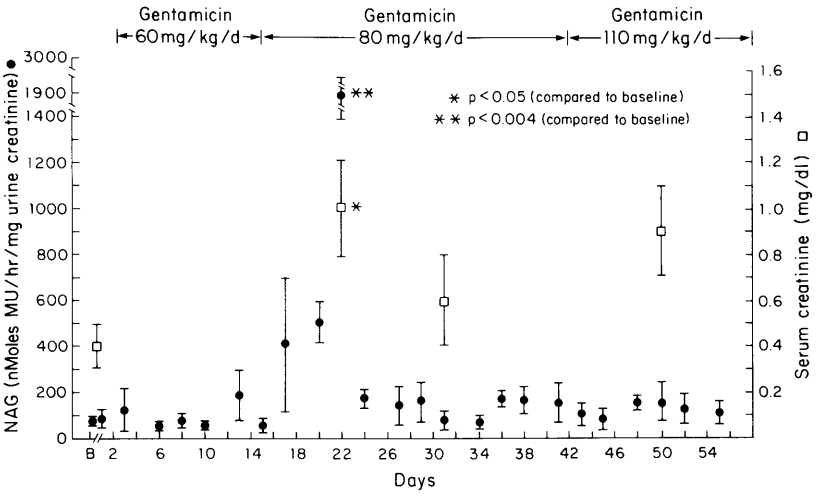

FIG. 3. Effect of 6 day per week gentamicin (40 mg/kg/day for 12 days, then 80 mg/kg/day for 30 days) injections on urinary *N*-acetyl-β-glucosaminidase (NAG) and serum creatinine levels (mean ± SD). On Day 42, a gentamicin challenge at 110 mg/kg 6 days per week was begun. $N = 7$.

The serum creatinine for all drug-treated groups, fell as NAG excretion returned to baseline.

Discussion. We have observed that rats given gossypol, spermine, or gentamicin develop an acute renal injury from which they apparently recover while continuing to receive the drug. They then appear resistant to the nephrotoxic effects of gentamicin.

While this transient nephrotoxicity caused by gentamicin has been previously observed (8), the observation of the same phenomenon during gossypol and spermine administration is new. A similar observation of transient renal injury with recovery during continuous gentamicin administration has been made by Trollfors in patients receiving gentamicin for treatment of chronic osteomyelitis (18). Of greater importance is our observation of the apparent cross-resistance of

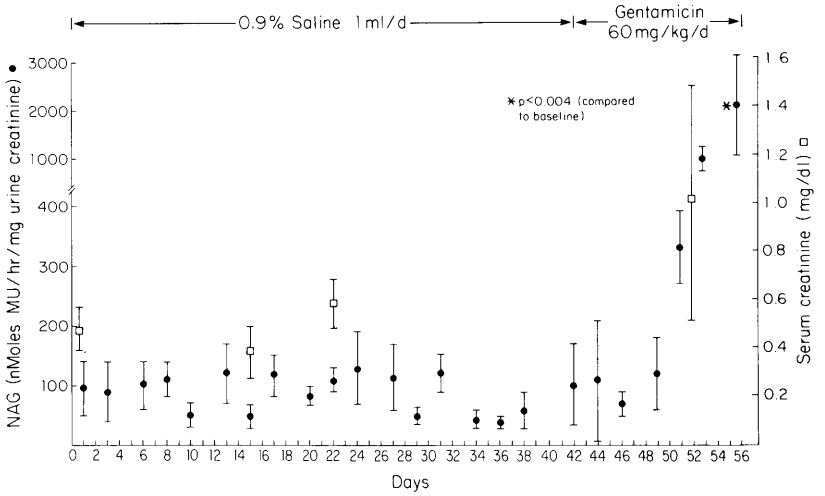

FIG. 4. Effect of 6 day per week saline injections (0.5 ml) on urinary *N*-acetyl-β-glucosaminidase (NAG) and serum creatinine levels (mean ± SD). On Day 42, a gentamicin challenge at 60 mg/kg 6 days per week was begun. $N = 8$.

spermine-adapted and gossypol-adapted rats to the nephrotoxicity of gentamicin. This indicates that a more general adaptive process is occurring than adaptation to the administered drug alone.

Gossypol is a polyphenolic compound. Spermine is an aliphatic amine and gentamicin is a polyamino antibiotic. There does not appear to be any similarity in structure of these three compounds to account for their cross-tolerance.

A mechanism that could account for the cross-tolerance would involve reactive oxygen species. Reactive oxygen species have been implicated in many human diseases (19) including renal diseases (20, 21). Reactive oxygen species have been incriminated in the renal injury from gentamicin (22, 23). While polyamines including spermine can absorb active oxygen radicals, under certain conditions spermine can increase the formation of reactive oxygen species (24, 25, 26). Gossypol increases reactive oxygen species produced by rat liver or kidney microsomes (27). Thus, all three of these compounds have, in common, the capacity to increase the amount of reactive oxygen species. From this, one can develop the hypothesis that the nephrotoxicity of each of these compounds is due to this enhanced oxidative effect. "Adaptation" then might follow the induction of enhanced "antioxidant" activity in the kidney. This hypothesis would account for cross-tolerance since the postulated mechanism of toxicity due to increased reactive oxygen species would be ameliorated by the enhanced antioxidant activity irrespective of which compound was given to induce the tolerance.

We are grateful to the staff of the Research Animal Resource Center at Cornell University Medical College and Dr. Hai T. Nguyen for their assistance.

1. Suzuki T, cited in Ref. (2).
2. MacNider, W deB. The functional and pathological response of the kidney in dogs subjected to a second subcutaneous injection of uranium nitrate. J Exp Med **49**:411–431, 1929.
3. Honda N, Hisida A, Ikuma K, Yoneumura K. Acquired resistance to acute renal failure. Kidney Int **31**:1233–1238, 1987.
4. Walzyck M, Houghton SC, Bennett WM. Selected aspects of experimental aminoglycoside nephrotoxicity. In: Bertani T, Remiczzi G, Garattini S, Eds. Drugs and Kidney. New York, Raven Press, pp. 95–106, 1986.
5. Gilbert DN, Houghton DC, Bennett WM, Plamp CE, Reger K, Porter GA. Reversibility of gentamicin nephrotoxicity in rats: Recovery during continuous drug administration. Proc Soc Exp Biol Med **160**:99–103, 1979.
6. Luft FC, Rankin LI, Slan KS, Yum MN. Recovery from aminoglycoside nephrotoxicity with continued drug administration. Antimicrob Agents Chemother **14**:284–287, 1978.
7. Elliot WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennett WM. Gentamicin nephrotoxicity I. Degree and permanence of acquired insensitivity. J Lab Clin Med **100**:501–512, 1982.
8. Elliott WC, Houghton DC, Gilbert DN, Baines-Hunter J, Bennet WM. Gentamicin nephrotoxicity II. Definitions of conditions necessary to induce acquired insensitivity. J Lab Clin Med **100**:513–525, 1982.
9. Rosenthal SM, Fisher ER, Stohlman EF. Nephrotoxic action of spermine. Proc Soc Exp Biol Med **80**:432–434, 1952.
10. Fisher ER, Rosenthal SM. Pathology and pathogenesis of spermine-induced renal disease. Arch Pathol **54**:244–253, 1954.
11. Restivo KS, Drayer DE, Orto L, Bond O, Reidenberg MM. The accumulation of polyamines and their weak association with lower body temperature in elderly convalescent patients. J Lab Clin Med **110**:217–220, 1987.
12. Wang YX, Chan ZX. The mechanism of gossypol leads to hypokalemia. International Symposium on Gossypol Research for Fertility Regulation. Wuhan, China, October 15–17, 1968. [Abstracts]
13. Heywood K, Lloyd GK, Majud SK, Gopinath C. The toxicity of gossypol to the male rat. Toxicology, **40**:279–284, 1986.
14. Shandilya L, Clarkson TB, Lewis JC. Effects of gossypol on reproductive and endocrine functions of male cynomolgus monkeys. Biol Reprod **27**:241–252, 1982.
15. Prescott LF. Assessment of nephrotoxicity. Brit J Clin Pharmacol **13**:303–311, 1982.
16. Merle LJ, Reidenberg MM, Camacho MT, *et al.* Renal injury in patients with rheumatoid arthritis treated with gold. Clin Pharmacol Ther **28**:216–222, 1980.
17. Leaback DH, Walker PB. Studies on glucosaminidase. 4: The fluorimetric assay of *N*-acetyl-β-glucosaminidase. Biochem J **78**:151–156, 1961.
18. Trollfors B. Gentamicin-associated changes in renal function reversible during continued treatment. J Antimicrob Chemother **12**:285–287, 1983.
19. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Ann Intern Med **107**:526–545, 1987.

20. Canavese C, Stratta P, Vercellone A. Oxygen free radicals in nephrology. Int J Artif Organs **10**:379–389, 1987.
21. Baud L, Ardaillou R. Reactive oxygen species: Production and role in the kidney. Amer J Physiol **251** (Renal Fluid Electrolyte Physiol **20**):F765–F776, 1986.
22. Ramsammy L, Ling KY, Josepovitz C, Levine R, Kaloyanides GJ. Effect of gentamicin on lipid peroxidation in rat renal cortex. Biochem Pharmacol **34**:3895–3900, 1985.
23. Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest **81**:334–341, 1988.
24. Gaugas JM, Dewey DL. Oxygen-dependent free radicals in spermine oxidation cytostasis and chemiluminescence and the role of superoxide dismutase. Brit J Cancer **41**:946–955, 1980.
25. Ferrante A, Rzepczyk CM, Saul AJ. Polyamine oxidase-mediated trypanosome killing: The role of hydrogen peroxide and aldehydes. J Immunol **133**:2157–2162, 1984.
26. Guarnieri C, Georgountzos A, Caldarera I, Flamigni F, Ligabue A. Polyamines stimulate superoxide production in human neutrophils activated by *N*-fMet-Leu-Phe but not by phorbol myristate acetate. Biochim Biophys Acta **930**:135–139, 1987.
27. Wu DF, Yu YW. Superoxide free radical formation stimulated by (±), (+), (−) gossypol in rat liver and kidney microsomes. Proc CAMS PUMC **1**(3):150–156, 1986.

Received March 21, 1988. P.S.E.B.M. 1988, Vol. 189.
Accepted September 2, 1988.