Biochemical Pharmacology, Vol. 36, No. 13, pp. 2125–2132, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

FAILURE OF INHIBITION OF LIPID PEROXIDATION BY VITAMIN E TO PROTECT AGAINST GENTAMICIN NEPHROTOXICITY IN THE RAT

LESLIE S. RAMSAMMY, CHRISTINE JOSEPOVITZ, KIT-YIN LING, BERNARD P. LANE and GEORGE J. KALOYANIDES*

Division of Nephrology and Hypertension, Department of Medicine, and Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794; and the Veterans Administration Medical Center, Northport, NY 11768, U.S.A.

(*Received* 13 *June* 1986; *accepted* 24 *November* 1986)

Abstract—We tested the hypothesis that accelerated lipid peroxidation, possibly at the level of the lysosome, is linked causally to the pathogenesis of aminoglycoside nephrotoxicity by investigating whether administration of vitamin E would inhibit lipid peroxidation and prevent or ameliorate gentamicin-induced proximal tubular cell injury. Five groups of rats were injected with either saline, vitamin E (600 mg/kg per day) for 6 days, gentamicin (100 mg/kg per day) for 6 days, vitamin E for 6 days plus gentamicin for 6 days or vitamin E for 12 days and gentamicin for the last 6 days. Gentamicin alone induced a 16% increase in renal cortical phospholipids; vitamin E had no significant effect on this change. Gentamicin alone caused accelerated lipid peroxidation evident by a doubling of renal cortical malondialdehyde to 1.23 nmol/mg protein, and a sharp decline of esterified polyunsaturated fatty acids, especially arachidonic acid which fell 43%. These changes were accompanied by depressions of superoxide dismutase, catalase, and total glutathione and a shift from reduced to oxidized glutathione. Concurrent treatment of rats with vitamin E plus gentamicin for 6 days had no significant effect on the gentamicin-induced alterations of malondialdehyde, superoxide dismutase, catalase or the glutathione cascade; however, the shift from polyunsaturated to saturated fatty acids was largely reversed. In rats pretreated with vitamin E for 6 days, gentamicin failed to raise renal cortical malondialdehyde above that of saline-treated rats. The changes in esterified fatty acids were prevented almost entirely, and there were no significant alterations from control of the glutathione cascade. The depressions of superoxide dismutase and of catalase, however, were not reversed. Vitamin E did not affect the amount of gentamicin accumulated in renal cortex nor did it prevent the gentamicin-induced rise of serum creatinine. Examination of renal cortex by light and electron microscopy revealed that vitamin E did not prevent or even reduce the severity of gentamicin-induced proximal tubular cell lesions and necrosis. These results confirm those we obtained in a previous study with the antioxidant diphenylphenylenediamine. The observation that inhibition of lipid peroxidation by two distinct antioxidants failed to prevent proximal tubular cell injury and renal dysfunction associated with gentamicin administration leads us to conclude that lipid peroxidation is a consequence and not a cause of gentamicin-induced nephrotoxicity.

We recently reported that gentamicin induces accelerated lipid peroxidation in the renal cortex of the rat [1]. Based on this observation we postulated that lipid peroxidation was a proximal event in the injury cascade of aminoglycoside nephrotoxicity. To test this hypothesis, rats were treated with diphenylphenylenediamine (DPPD†), an antioxidant [2]. This compound proved to be highly effective in preventing accelerated lipid peroxidation but it did not abrogate gentamicin-induced nephrotoxicity [3]. These findings support the view that lipid peroxidation is a consequence rather than a cause of aminoglycoside-induced proximal tubular cell injury. Although DPPD is effective in preventing lipid peroxidation as assessed in terms of gross changes measured in a homogenate of renal cortex, we cannot exclude the possibility that this compound may have failed to inhibit lipid peroxidation at a critical intracellular site perhaps due to inadequate access to that site. For example, aminoglycoside nephrotoxicity is characterized by congestion of the lysosomal compartment with phospholipid in the form of myeloid bodies [4–6] which is thought to be a consequence of drug-induced inhibition of lysosomal phospholipase degradation of phospholipid [7, 8]. We have shown that the increased lysosomal content of phospholipid is enriched in phosphatidylinositol [4]. Since arachidonic acid is typically found in the sn-2-position of phosphatidylinositol [9], the accumulation of this phospholipid within lysosomes could serve as substrate for free radical attack [10, 11]. If DPPD did not gain access to the lysosomal compartment, this reaction could have proceeded at a time when other lipid peroxidation reactions at other subcellular sites had been depressed or completely inhibited. Moreover, given the small size of the lysosomal compartment, persistence of lipid peroxidation within lysosomes might not be readily detected as a change from baseline of the various parameters monitored.

* Correspondence to: George J. Kaloyanides, M.D., Division of Nephrology and Hypertension, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794.

Accelerated lipid peroxidation at the level of the lysosome has been shown to cause lysis of the lysosomal membrane [12–15]. This finding is of particular interest because several groups of investigators have implicated labilization of lysosomes in the pathogenesis of aminoglycoside-induced proximal tubular cell injury [16–19].

Vitamin E is a lipid soluble antioxidant which distributes preferentially to membranes enriched in polyunsaturated fatty acids [20–22] and is highly susceptible, therefore, to lipid peroxidation [10, 11]. Vitamin E has been demonstrated to protect against peroxidative injury in a number of experimental settings [12, 23–28]. Importantly, vitamin E has been shown to be effective in blocking the induction of lysosomal lipid peroxidation and reducing the loss of lysosomal latency [12, 15]. Therefore, we undertook the present studies to investigate the efficacy of vitamin E in preventing lipid peroxidation and proximal tubular cell injury in rats treated with the nephrotoxic aminoglycoside gentamicin.

METHODS

Experiments were performed in five groups of male Sprague–Dawley rats (weighing 200–250 g) injected subcutaneously with: (1) 0.9% NaCl for 6 days; (2) vitamin E in ethanol, 600 mg/kg per day for 6 days; (3) gentamicin sulfate, 100 mg base/kg per day for 6 days; (4) vitamin E, 600 mg/kg per day for 6 days and gentamicin sulfate, 100 mg/kg per day for 6 days; (5) vitamin E, 600 mg/kg per day for 12 days and gentamicin, 100 mg/kg per day for 6 days (days 7–12). Vitamin E and gentamicin were injected separately. Twenty-four hours after the last injection, rats were killed under pentobarbital anesthesia by exsanguination from the aorta. The renal cortex was rapidly dissected free and homogenized in 0.9% NaCl buffered to pH 7.4 with sodium phosphate. Aliquots of homogenate were processed for subsequent measurements of phospholipids, malondialdehyde (MDA), fatty acids, glutathione, catalase, superoxide dismutase (SOD), protein and gentamicin.

Renal cortical phospholipids were extracted in chloroform-methanol (2:1, v:v) containing 0.01 M tetrabutylammonium sulfate and were quantitated by inorganic phosphorus determination [29]. Fatty acids were removed from phospholipids by alkaline hydrolysis with simultaneous formation of methyl ester derivatives and analyzed by GLC as previously described [1]. MDA was determined by the thiobarbituric acid procedure [30]. Glutathione was assayed by monitoring spectrophotometrically the reduction of 5,5-dithiobis-2-nitrobenzoic acid by NADPH [31]. Catalase activity was determined by the method of Cohen [32] which involves the titration of $KMnO_4$. The activity of catalase was expressed as the first-order reaction rate constant, k, which is defined by the equation $k = \log (S_o/S_t) \times 2.3/t$ where t = time, and S_o = initial concentration of $KMnO_4$ at time t. Superoxide dismutase (SOD) was determined on the 700 g supernatant fraction of renal cortical homogenate containing both cytosolic and mitochondrial enzyme and was measured as the rate of inhibition of ferricytochrome c reduction in a xanthine–xanthine oxidase generating system [33]. One unit of SOD is defined as that quantity required to inhibit the rate of reduction of ferricytochrome c by 50%. Protein was determined by the method of Lowry *et al.* [34]. Gentamicin in renal cortex was measured using the Emit Gentamicin Assay (Sylva Co., Palo Alto, CA). Serum creatinine was measured by the method of Mitchell [35] which involves column chromatography to separate true creatinine from chromagen.

Kidneys of four rats from groups 1, 3 and 5 were perfused *in situ* with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer at pH 7.3. Ten representative blocks of tissue (1 mm^3) were taken from the inner cortex and outer cortex of one kidney of each rat. The blocks were treated with an unbuffered aqueous solution of osmium tetroxide, dehydrated with alcohol, and embedded in Epon 812. All blocks were sectioned for light microscopy. The sections were stained with Azure II-methylene blue and coded so that the observer (B. L.) could not distinguish control from experimental material. Each section was scored for the presence and site of necrosis, vacuolization and cytologic inclusions. Replicate sections from the same kidney were evaluated for uniformity. Thin sections for electron microscopy were cut from blocks from each cortical zone. The faces were trimmed to central regions in which tubules were cut in cross sections and glomeruli were absent. Electron micrographs were taken at 3000 diameters of at least four proximal tubules from each zone of each kidney. A square grid was placed on the micrographs, and the area of cytoplasm occupied by lysosomes and the area occupied by myeloid bodies were measured independently using standard morphometric methods [36].

The data were subjected to analysis of variance and the Duncan multiple range test to define stat-

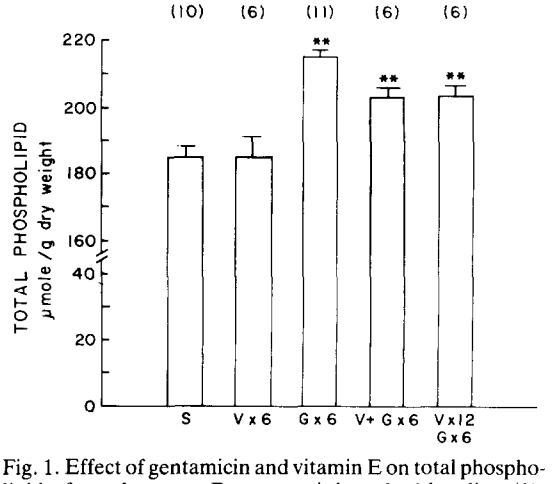

Fig. 1. Effect of gentamicin and vitamin E on total phospholipid of renal cortex. Rats were injected with saline (S), gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E (V) 600 mg/kg per day for 6 or 12 days. The numbers in parentheses at the top of the bars represent the number of rats in each group. The bars represent the mean ± SE. Key: (**) significantly different from saline, $P < 0.01$.

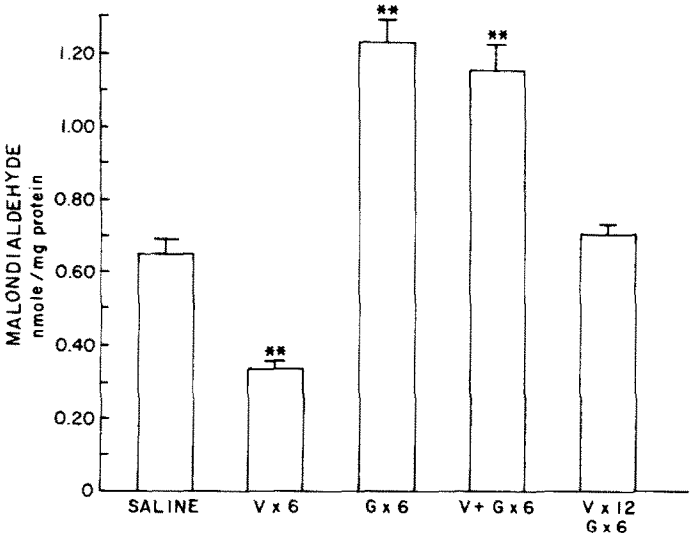

Fig. 2. Effect of gentamicin and vitamin E on malondialdehyde of renal cortex. Rats were injected with saline (S), gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E (V) at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline $P < 0.01$.

istically significant differences. The results in the text and figures are expressed as the mean ± SEM.

RESULTS

Vitamin E alone had no effect on total phospholipids of renal cortex (Fig. 1). In gentamicin-treated rats, renal cortical phospholipids were increased significantly above that of control rats. Treatment of rats with vitamin E for 6 or 12 days did not prevent the gentamicin-induced renal cortical phospholipidosis.

MDA, an end product of lipid peroxidation, was found to be depressed in the renal cortex of rats treated with vitamin E alone compared to that of saline-injected control rats (Fig. 2). In gentamicin-injected rats, MDA increased 89% above that of control rats ($P < 0.01$), and this increase was not

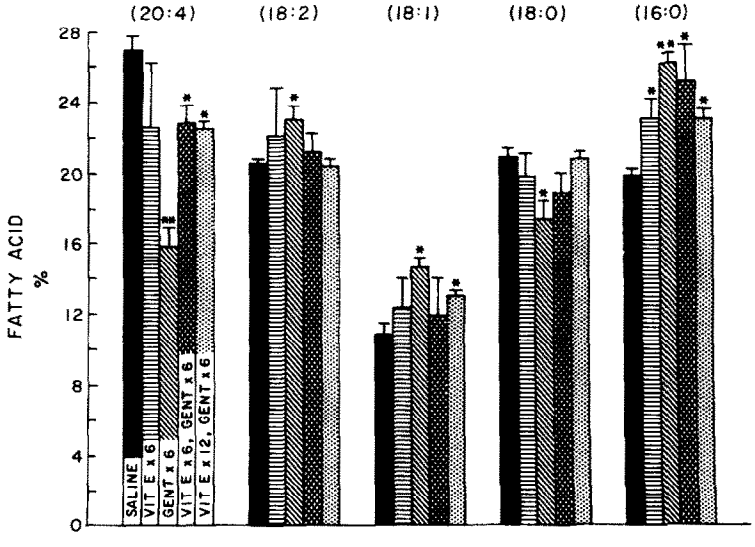

Fig. 3. Effect of gentamicin and vitamin E on fatty acid composition of renal cortical phospholipid. Rats were injected with saline, gentamicin (GENT) at 100 mg/kg per day for 6 days and/or vitamin (VIT) E at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. The numbers above the bars indicate the number of carbon atoms followed by the number of double bonds. Key: 20:4, arachidonic acid; 18:2, linoleic acid; 18:1, oleic acid; 18:0, stearic acid; 16:0, palmitic acid; (*) significantly different from saline, $P < 0.05$; and (**) significantly different from saline. $P < 0.01$.

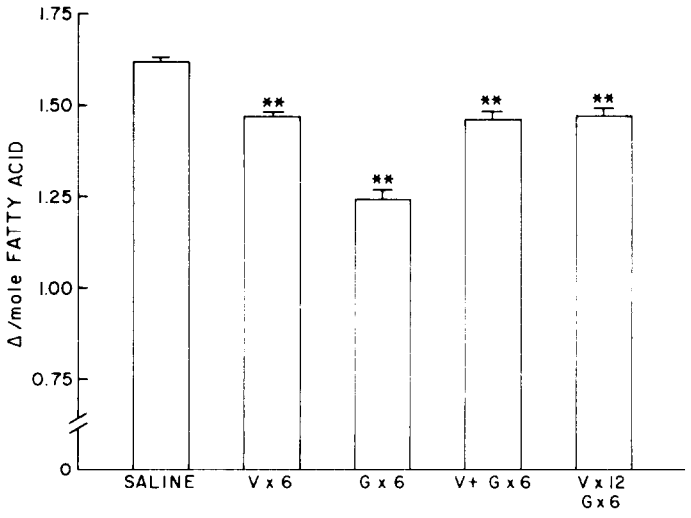

Fig. 4. Effect of gentamicin and vitamin E on the average number of double bonds (Δ) per mole fatty acid esterified in renal cortical phospholipids. Rats were injected with saline, gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E (V) at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline, $P < 0.01$.

attenuated significantly by 6 days of vitamin E therapy. In rats given 12 days of vitamin E therapy, MDA was not different from that of saline-injected rats.

The shift from polyunsaturated to saturated fatty acids provides another sensitive indicator of lipid peroxidation. These data are summarized in Fig. 3. In rats injected with vitamin E alone, there was a slight shift from polyunsaturated towards saturated fatty acids compared to that observed in saline-injected rats, but only in the case of the saturated fatty acid palmitate was the change statistically significant. Rats injected with gentamicin manifested a significant decline (−43%) of arachidonic acid (AA) compared to that of saline-injected rats ($P < 0.01$) and of rats injected with vitamin E alone ($P < 0.05$). The decline of AA was accompanied by significant increases of linoleic, oleic, and palmitic acids and a significant decrease of stearic acid. This pattern of change is similar to our previously reported observations [1, 3]. In rats injected with gentamicin plus vitamin E for 6 or 12 days, these changes in the composition of fatty acids esterified in renal cortical phospholipids were either entirely or largely prevented.

Calculation of the average number of double bonds (Δ) per mole of fatty acid reveals that in rats injected with vitamin E alone Δ/mole fatty acid declined slightly, whereas it was greatly depressed in rats treated with gentamicin (Fig. 4). In rats injected with gentamicin plus vitamin E, Δ/mole fatty acid was not different from that of rats injected with vitamin E alone.

Figure 5 summarizes the SOD data. Treatment with vitamin E alone was associated with depression of SOD compared to that of saline-injected rats. In

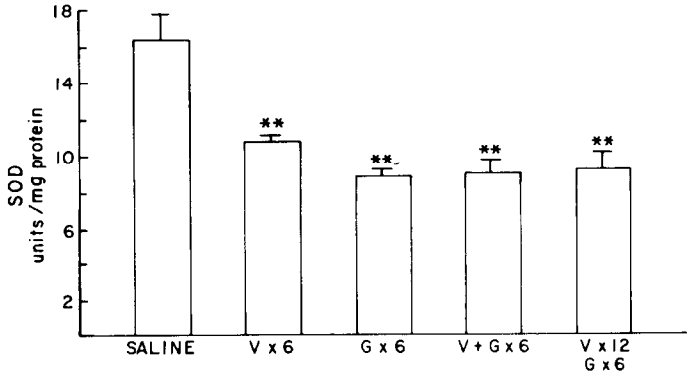

Fig. 5. Effect of gentamicin and vitamin E on superoxide dismutase (SOD) in renal cortex. Rats were injected with saline, gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E (V) 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline, $P < 0.01$.

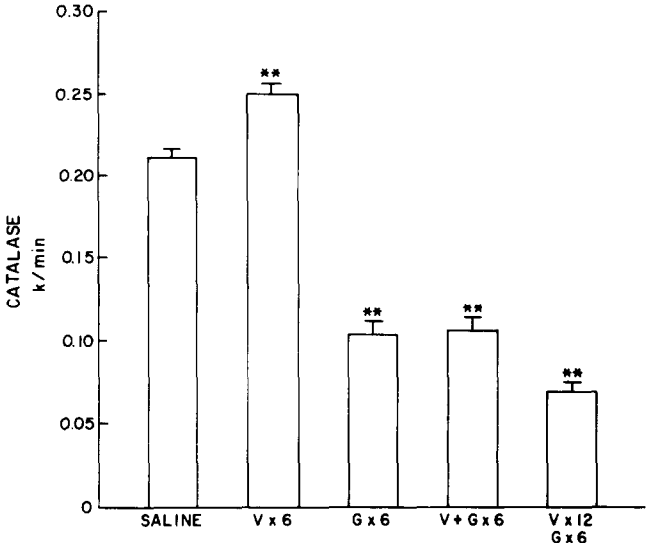

Fig. 6. Effect of gentamicin and vitamin E on catalase activity in renal cortex. Rats were injected with saline, gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E (V) at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline. $P < 0.01$.

contrast to our previous reports [1, 3], gentamicin treatment also depressed SOD, albeit only slightly, and this effect was not influenced by vitamin E.

In contrast to the effect on SOD, vitamin E alone was associated with an increase of catalase activity compared to that of saline-injected rats (Fig. 6). In agreement with our earlier findings [1, 3], rats injected with gentamicin exhibited depression of catalase, and this effect was not prevented by the addition of vitamin E.

Vitamin E alone has no significant effects on total glutathione, GSH, or GSSG (Fig. 7). Six days of gentamicin therapy resulted in depression of total and reduced glutathione and increased oxidized glutathione and the ratio of oxidized to total glutathione. These changes confirm our earlier observations [1, 3]. Injection of vitamin E for 6 days concurrently with gentamicin did not alter the effects of gentamicin on the glutathione cascade, whereas pretreatment with vitamin E completely prevented gentamicin-induced alterations of the glutathione cascade.

Vitamin E did not affect the amount of gentamicin accumulated in the renal cortex (Fig. 8). The concurrent administration of vitamin E with gentamicin did not prevent the gentamicin-induced rise of the serum creatinine concentration (Fig. 9).

Histopathology. Sections stained by light micro-

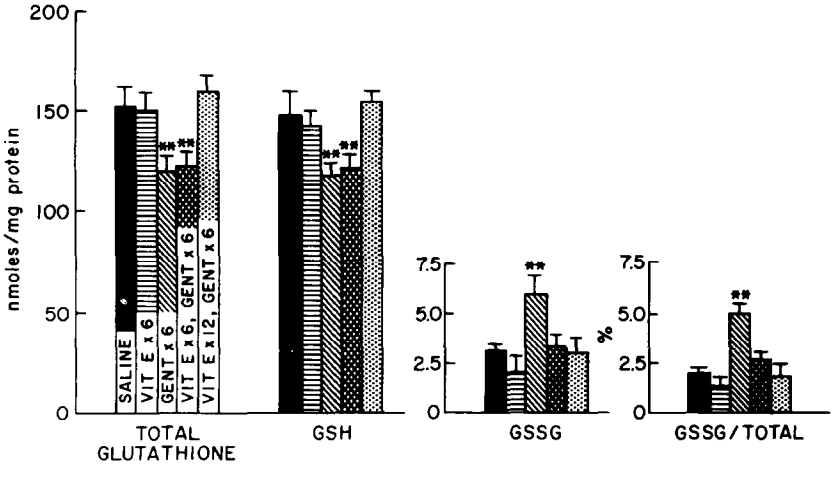

Fig. 7. Effect of gentamicin and vitamin E on total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in renal cortex. Rats were injected with saline, gentamicin (GENT) at 100 mg/kg per day for 6 days and/or vitamin (VIT) E for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline, $P < 0.01$.

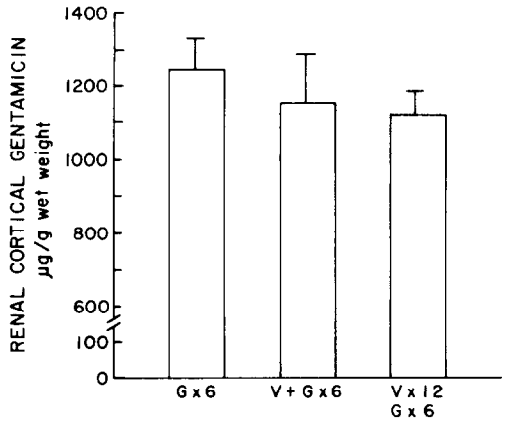

Fig. 8. Effect of vitamin E on the renal cortical concentration of gentamicin. Rats were injected with gentamicin (G) at 100 mg/kg per day for 6 days and vitamin E at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE.

scopy revealed patchy single cell necrosis or dropout in proximal tubules of kidneys from all gentamicin-treated rats. The majority of necrotic cells were in the pars recta. Approximately 7% of proximal tubular cells were necrotic in kidneys of rats injected with gentamicin plus vitamin E compared to less than 2% of cells in kidneys or rats injected only with gentamicin. Large irregularly stained cytoplasmic inclusions were present in proximal tubule cells in light microscopic plastic sections from rats injected with gentamicin or gentamicin plus vitamin E. In the inner cortex, 50–70% of proximal tubule cells and in the outer cortex 20–30% of proximal tubule cells contained inclusions. There was substantial variability in the numbers of inclusions among cells of the same tubule as well as among cells of different tubules so that quantitation of inclusions on a per cell basis was not done. Sections of control kidneys exhibited no necrosis and only rare blue-stained inclusions.

At the ultrastructural level most of the inclusions in proximal tubule cells were irregularly shaped membrane bound cytoplasmic areas with finely particulate, moderately dense matrix and one or more myeloid bodies. The inclusions occupied 26% (range 8–39%) of the cytoplasm of pars recta cells of gentamicin-injected rats and 20% (range 6–33%) of the cytoplasm of comparable cells in gentamicin–vitamin E treated animals. Myeloid bodies occupied approximately 10% (range 6–24%) of the cytoplasm of rats injected with gentamicin and 8% (range 4–18%) of the cytoplasm of rats treated with gentamicin and vitamin E.

DISCUSSION

The results of these experiments confirm our previous studies [1, 3] that gentamicin treatment of rats causes accelerated lipid peroxidation in the renal cortex as reflected by increased MDA, an end product of lipid peroxidation, and by depression of polyunsaturated fatty acids, which serve as substrate for free radical attack [10, 11]. Moreover, we detected alterations in the three major systems which normally function to protect the cell against free radical attack. The activities of SOD and catalase were depressed which raises the possibility that impaired removal of free radicals by these antioxidant systems may have contributed to the accelerated lipid peroxidation [10, 11]. The glutathione system manifested a shift from reduced to oxidized glutathione, a response pattern consistent with increased oxidative stress [10, 11, 37]. The accelerated lipid peroxidation and alterations in the antioxidant systems were accompanied by functional and histopathologic evidence of gentamicin nephrotoxicity. Serum creatinine concentration was elevated significantly which signifies depression of glomerular filtration rate, and proximal tubular cell injury and necrosis were observed by light and electron microscopy.

To assess the role of lipid peroxidation in the pathogenesis of gentamicin-induced proximal tubular cell injury, rats were treated with a large dose of

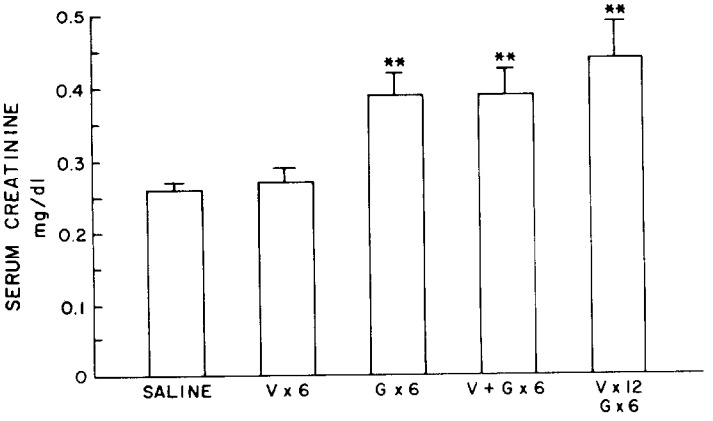

Fig. 9. Effect of gentamicin and vitamin E on the serum creatinine concentration. Rats were injected with saline, gentamicin (G) at 100 mg/kg per day for 6 days and/or vitamin E at 600 mg/kg per day for 6 or 12 days. The bars represent the mean ± SE. Key: (**) significantly different from saline, $P < 0.01$.

vitamin E, an antioxidant which has been shown to scavenge free radicals and inhibit lipid peroxidation *in vitro* [12, 27, 28] and *in vivo* [12, 23–27]. The major effect of vitamin E when administered alone to rats was to depress basal MDA. A slight decline of polyunsaturated fatty acids was also observed. When vitamin E was administered concurrently with gentamicin, its effects on the parameters of lipid peroxidation were mixed. For example, vitamin E had no discernible effect on gentamicin-induced elevation of MDA, or the depression of SOD, catalase, and total and reduced glutathione. However, it largely prevented or reversed the alterations in the composition of fatty acids esterified in renal cortical phospholipids. The failure of vitamin E to prevent gentamicin-induced lipid peroxidation when administered simultaneously with gentamicin could reflect failure to achieve adequate concentrations of vitamin E at critical subcellular membranes using this dose and time schedule. To circumvent this problem, we pretreated rats with vitamin E for 6 days before challenging them with gentamicin. We reasoned that during this time interval vitamin E would be taken up into cellular membranes, particularly those enriched in polyunsaturated fatty acids [20–22] and most susceptible to lipid peroxidation [10, 11], and would facilitate the delivery of vitamin E to the lysosomal compartment.

Pretreatment with vitamin E was effective in preventing MDA from rising above the level of control rats. It almost completely restored the alterations in the composition of fatty acids esterified in renal cortical phospholipids. It prevented alterations in the glutathione cascade. In contrast, vitamin E did not reverse the depression of SOD or of catalase induced by gentamicin. In the case of catalase, the activity was further depressed in rats pretreated with vitamin E. In our previous study, we observed, that DPPD also prevented the changes in MDA, fatty acid composition and glutathione but it failed to restore catalase activity [3]. It is uncertain to what extent the depression of catalase reflects a direct interaction between gentamicin and the enzyme. In any event, pretreatment with vitamin E inhibited, to an impressive degree, accelerated lipid peroxidation in rats injected with gentamicin. Nevertheless, this antioxidant did not abrogate or even ameliorate gentamicin-induced proximal tubular cell injury as assessed by light and electron microscopy. In fact, the degree of proximal tubular cell necrosis tended to be greater in the vitamin E treated rats. In addition, vitamin E pretreatment afforded no protection against depression of glomerular filtration as assessed by measurement of the serum creatinine concentration. The failure of vitamin E to protect against gentamicin-induced nephrotoxicity cannot be explained by differences in the accumulation of drug by proximal tubular cells. The renal cortical concentrations of gentamicin were similar in the three gentamicin-treated groups.

The objective of the present study was to evaluate the hypothesis that accelerated lipid peroxidation at the level of the phospholipid-ladened lysosomal compartment might be a proximal event in the injury cascade induced by gentamicin. Although we did not directly measure lipid peroxidation within lysosomes, it is inconceivable that the pretreatment protocol involving large doses of vitamin E would not have had some inhibitory effect on lysosomal lipid peroxidation. The fact that vitamin E did not even ameliorate gentamicin nephrotoxicity leads us to reject our hypothesis that accelerated lipid peroxidation at the level of the lysosomes is a proximal event in the injury cascade induced by aminoglycosides.

The results of the present study confirm those obtained in our previous study with DPPD. Inhibition of lipid peroxidation by two distinct antioxidants failed to prevent proximal tubular cell injury and necrosis associated with gentamicin administration. We conclude, therefore, that lipid peroxidation is a consequence and not a cause of gentamicin-induced nephrotoxicity.

Acknowledgements—This work was supported by research grant AM 27061 from the ADDKD Institute of the National Institutes of Health and by a research grant from the Research Service of the Veterans Administration.

REFERENCES

1. L. Ramsammy, K-Y. Ling, C. Josepovitz, R. Levine and G. J. Kaloyanides, *Biochem. Pharmac.* **34**, 3895 (1985).
2. S. O. Wells, *J. Nutr.* **109**, 1673 (1979).
3. L. S. Ramsammy, C. Josepovitz, K-Y. Ling, B. P. Lane and G. J. Kaloyanides, *J. Pharmac. exp. Ther.* **238**, 83 (1986).
4. C. Josepovitz, T. Farruggella, R. Levine, B. Lane and G. J. Kaloyanides, *J. Pharmac. exp. Ther.* **235**, 810 (1985).
5. G. Aubert-Tulkens, F. Van Hoof and P. Tulkens, *Lab. Invest.* **40**, 481 (1979).
6. R. A. Giuliano, G. J. Paulus, G. A. Verpooten, V. M. Pattyn, D. E. Pollet, E. J. Nouwen, G. Laurent, M. B. Carlier, P. Maldague, P. Tulkens and M. E. DeBroe, *Kidney Int.* **26**, 838 (1984).
7. G. Laurent, M. B. Carlier, B. Rollman, F. Van Hoof and P. Tulkens, *Biochem. Pharmac.* **31**, 3861 (1982).
8. K. Y. Hostettler and L. B. Hall, *Proc. natn. Acad. Sci. U.S.A.* **79**, 1663 (1982).
9. B. J. Holub, *A. Rev. Nutr.* **6**, 563 (1986).
10. B. Chance, H. Sies and A. Boveris, *Physiol. Rev.* **59**, 527 (1979).
11. R. F. del Maestro, *Acta physiol. scand.* Suppl. 492, 153 (1980).
12. A. L. Tappel, *Fedn. Proc.* **24**, 73 (1965).
13. K. L. Fong, P. B. McCay and J. L. Poyer, *J. biol. Chem.* **248**, 7792 (1973).
14. T. Mak, H. P. Misra and W. B. Weglicki, *J. biol. Chem.* **258**, 13733 (1983).
15. W. B. Weglicki, B. F. Dickens and I. T. Mak, *Biochem. biophys. Res. Commun.* **124**, 229 (1984).
16. P. M. Tulkens, M. E. DeBroe, P. Maldague and J. A. Henson-Steinnow, in *Acute Renal Failure* (Eds. K. Solez and A. Whelton), p. 299. Marcel Dekker, New York (1983).
17. J. H. Powell and M. M. Reidenberg, *Biochem. Pharmac.* **31**, 3447 (1982).
18. J. P. Morin, G. Viotte, A. Vanderwalle, F. Van Hoof and P. Tulkens, *Kidney Int.* **18**, 583 (1980).
19. J. P. Fillastre, J. M. Kuhn, J. P. Bendirdjian, B. Foucher, J. P. Le Seur, P. Rollin and R. Vaillant, in *Advances in Nephrology* (Eds. J. Hamburger, J. Crosnier and M. H. Maxwell), p. 343. Year Book Medical Pubs., Chicago (1976).

20. I. Molennaar, C. E. Halstaert and M. J. Hardonk, in *Vitamin E, A Comprehensive Treatise* (Ed. L. J. Macklin), p. 372. Marcel Dekker, New York (1980).
21. J. A. Lucy, in *Tocopherol, Oxygen and Biomembranes* (Eds. C. De Duve and O. Hayashi), p. 109. Elsevier/North-Holland Biomedical Press, Amsterdam (1978).
22. A. T. Diplock, in *Biology of Vitamin E* (Ciba Foundation Symposium 101), p. 45. Pitman, London (1983).
23. M. M. Giblert, J. Sprecher, L. W. Chang and L. F. Meisner, *J. Toxic. environ. Hlth* **12**, 767 (1983).
24. L. J. Willmore and J. J. Rubin, *Expl. Neurol.* **83**, 62 (1984).
25. F. W. Summerfield and A. L. Tappel, *Mutation Res.* **126**, 113 (1984).
26. S. Addya, K. Chakravarti, A. Basu, M. Santra, S. Haldar and G. C. Chatterjee, *Acta vitamin. enzym.* **6**, 103 (1984).
27. P. B. McCay, *A. Rev. Nutr.* **5**, 323 (1985).
28. K. Fukuzawa, S. Takase and H. Tsukatari, *Archs Biochem. Biophys.* **240**, 117 (1985).
29. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
30. H. Ohkawa, N. Ohishi and K. Yagi, *Analyt. Biochem.* **95**, 351 (1979).
31. F. Tietze, *Analyt. Biochem.* **27**, 502 (1969).
32. G. Cohen, *Analyt. Biochem.* **34**, 30 (1970).
33. J. M. McCord and I. Fridovich, *J. biol. Chem.* **244**, 6049 (1969).
34. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
35. R. J. Mitchell, *Clin. Chem.* **19**, 408 (1973).
36. E. R. Weibel, *Int. Rev. Cytol.* **26**, 235 (1969).
37. C. V. Smith, H. Hughes, B. H. Lauterburg and J. R. Mitchell, in *Functions of Glutathione: Biochemical, Physiological and Clinical Aspects* (Eds. A. Laarson, S. Orrenius, A. Holmgren and B. Manervik), p. 125. Raven Press, New York (1983).