Insulin Reverses the Protection Given by Diabetes Against Gentamicin Nephrotoxicity in the Rat (43785)

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Abstract. **Rats with untreated diabetes mellitus are protected from gentamicin-induced nephrotoxicity. In order to evaluate the role of hyperglycemia, glycosuria, and polyuria in this phenomenon, miniosmotic pumps filled with insulin were implanted for 15 days in seven female Sprague-Dawley rats with streptozotocin-induced diabetes mellitus. Plasma glucose levels were successfully maintained under 126 mg/dl. To serve as the control group, eight age-matched diabetic (plasma glucose >400 mg/dl) rats had miniosmotic pumps placed delivering only Ringer's solution. Six days after placement of the pumps, gentamicin (40 mg/Kg/day) was administered to all animals for 9 days. The insulin-treated diabetic rats exhibited clear signs of nephrotoxicity by Day 6 of gentamicin, whereas the diabetic control group remained free from any functional or morphological evidence of proximal tubular damage throughout the 9 days of the aminoglycoside administration. At the end of the experiment, the creatinine clearance in the insulin-treated diabetic group was 45% lower than in the untreated diabetic group ($P < 0.005$). In addition, there was a rise in plasma creatinine ($P < 0.02$), muramidase appeared in the urine, and mild patchy acute tubular necrosis of the renal cortex was observed by light microscopic examination. The insulin-treated group also accumulated more gentamicin in the renal cortex than the untreated animals ($P < 0.005$). It is concluded that protection against the nephrotoxic effects of gentamicin is a feature of untreated experimental diabetes mellitus in the rat and that correction of the hyperglycemic state with insulin reverses this resistance.**

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Studies from our laboratory have shown that, in contrast to nondiabetic animals, the rat with streptozotocin-induced diabetes mellitus (DM) is protected against the nephrotoxic effects of gentamicin (1). Other investigators have subsequently also confirmed the resistance of DM rats to gentamicin-induced nephrotoxicity (2, 3). This resistance is associated with decreased accumulation of gentamicin in the renal cortex (1, 4), appears as early as 5 days after induction of diabetes (4), it is not influenced by its duration (4), and is not related to high urine flow or solute excretion rate, since nondiabetic rats with hereditary diabetes insipidus (1) or renal glycosuria induced by phlorizin (5) are unprotected against gentamicin-induced nephrotoxicity.

We have observed that when DM rats spontaneously lose their diabetic state they become susceptible to gentamicin-induced nephrotoxicity (6). Furthermore, when DM rats were partially treated with insulin to match the moderate osmotic diuresis induced by phlorizin (5), a minimal degree of renal damage was evident, suggesting that the attenuation of the diabetic state could obviate the level of the protection against gentamicin-induced nephrotoxicity observed in untreated diabetic rats (1, 4).

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The purpose of the current study was to assess whether control of hyperglycemia, glycosuria, and polyuria changed the response of DM rats to gentamicin. After 9 days of aminoglycoside administration, nephrotoxicity was demonstrated only in the insulin-treated DM animals, whereas the hyperglycemic glycosuric DM group remained free from renal damage.

Materials and Methods

Fifteen adult female Sprague-Dawley rats (160–270 g) (CD strain; Charles River Breeding Laboratory, Wilmington, MA) with streptozotocin-induced diabetes mellitus were placed in individual metabolic cages, fed a standard rat chow, and given free access to tap water. Diabetes was induced 6 months prior to the beginning of the study by the iv administration of streptozotocin at a dose of 65 mg/kg body wt. The diabetic state was defined as persistently elevated nonfasting plasma glucose levels (>400 mg/dl), high degree of glycosuria (>5 g/day), and marked polyuria (daily urine volume >60 ml/day).

Figure 1 illustrates the protocol of the study. After 1 week of adaptation, the animals were divided into two groups: the insulin-treated group (DM-INS, n = 7), and the untreated diabetic rats (DM-UNR$_X$, n = 8) which served as controls. In the DM-INS group, miniosmotic pumps, model 2002 (Alza Co., Palo Alto, CA) filled with U-500 regular insulin (Eli Lilly and Co., Indianapolis, IN) and 0.25 μmol of aspartic and glutamic acid (7) were implanted subcutaneously in the interscapular area, and set to deliver 6 units of regular insulin per day for 15 days at a rate of 0.5 μl/hr. This dose was selected after preliminary experiments established it as the optimal dose to control glucose levels and is similar to that previously reported by others (8). Pumps filled with Ringer's solution, delivering at a rate of 0.5 μl/hr, were implanted in the DM-UNR$_X$ group,

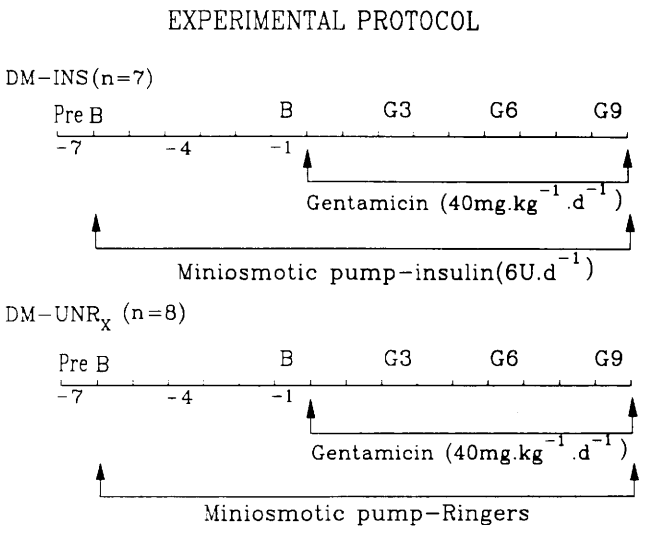

Figure 1. Experimental protocol.

as described above. Six days after the placement of the pumps, all animals began to receive gentamicin sulfate (Schering Co., Kenilworth, NJ), in equally divided doses (7:00 AM and 3:00 PM), given subcutaneously, for a total daily dose of 40 mg/kg, during 9 days. The gentamicin dosage was adjusted for body weight changes three times during the experiments.

Metabolic studies were conducted prior to the placement of the miniosmotic pumps (Prebaseline, PRE B), on Day 5 and 6 of insulin or sham (Ringer's) therapy (Baseline, B), and on Day 3 (G3), 6 (G6), and 9 (G9) of gentamicin administration (Fig. 1). On these days, the rats were weighed, and their water and food intake measured. Twenty-four-hour urine collections were obtained under mineral oil, and blood was sampled from their tails at the end of the urine collection periods, as previously described (1). After the completion of Day G9, the animals were anesthetized with pentobarbital, and their kidneys perfused via the aorta with Ringer's solution. The vessels to the right kidney were clamped and the kidney removed for determination of gentamicin. The left kidney was then perfused *in situ* with Karnovsky's fixative and subsequently removed for light microscopy examination.

Glomerular filtration rate was estimated in this study by the 24-hr urine endogenous creatinine clearance using true creatinine measurements in blood and urine (1). Concern may arise, however, about the possibility that diabetes may increase the tubular secretion of creatinine leading to an increase in the calculated creatinine clearance and thus, minimizing or, masking a decline in glomerular filtration rate after gentamicin in these animals (3). We have recently demonstrated that the ^{14}C inulin clearance (CIN) remained unchanged in female diabetic rats receiving gentamicin for 14 days, whereas control rats developed a marked decrease in glomerular filtration rate (9). Furthermore, the simultaneously measured creatinine and inulin clearance ratios remained essentially unity and unchanged: at baseline (0.98), and after Days 9 (1.03) and 14 (1.02) of gentamicin administration (9). This observation makes it extremely unlikely that, at least in the female Sprague-Dawley rat, the diabetic state may interfere with the tubular secretion of creatinine.

The analytical methods and calculations have been previously described (1). Blood and urine glucose were measured with a Beckman Glucose Analyzer, and urinary protein with a microadaptation of the biuret method. The renal cortical content of gentamicin was measured in duplicate by an enzymatic radiochemical assay (^{14}C gentamicin; P. L. Biochemical, Inc., Milwaukee, WI) and the results expressed as μg/g of wet kidney tissue. For this measurement the kidneys were obtained on Day 10 at least 16 hr after the last dose of gentamicin was given (10). Proximal

tubular damage was evaluated histologically with coded slides by one of us (V.P.) and graded from 0 to 4 according to the following criteria: 0 = no necrosis; 1 = single cell necrosis in sparse tubules; 2 = sparse tubules showing more than one cell involved; 3 = tubules in almost every high power field exhibiting necrosis; and, 4 = total necrosis. For each group, the results were averaged and expressed as a tubular necrosis score.

Values are presented as means ± SEM. Statistical significance of differences within and between groups was assessed by analysis of variance with repeated measures using the Multivariate General Linear Hypothesis program in a SYSTAT statistical package. The same program was used for regression analysis. When appropriate, Student's *t* test or a nonparametric test (Mann-Whitney) was used to compare differences between groups.

Results

Prebaseline. All animals featured body weights lower than those expected for nondiabetic female rats of similar age (Fig. 2). In addition, high urine volume, high nonfasting plasma glucose concentration, and high degree of glycosuria were present, owing to the 6-month duration of their diabetes. More importantly, the severity of diabetes and the level of renal function were similar in both groups (Table I).

Baseline. During this phase, the DM-INS rats rapidly lost the diabetic features of hyperglycemia, glycosuria, and high urine volume. Body weight in this group increased an average of 33 g, whereas the increment was only 5 g in the DM-UNRx animals. These changes were statistically different from each other (Fig. 2). In comparison to PRE B, however, there were no changes in food intake, and urine osmolality, and no significant differences were noted in plasma creatinine and endogenous creatinine clearance (Ccr) (Table I). On the other hand, the DM-UNRx rats maintained all the features exhibited at the PRE B stage. It is noteworthy that at the end of this period the level of kidney function as measured by plasma creatinine and Ccr, was identical for both groups (Table I).

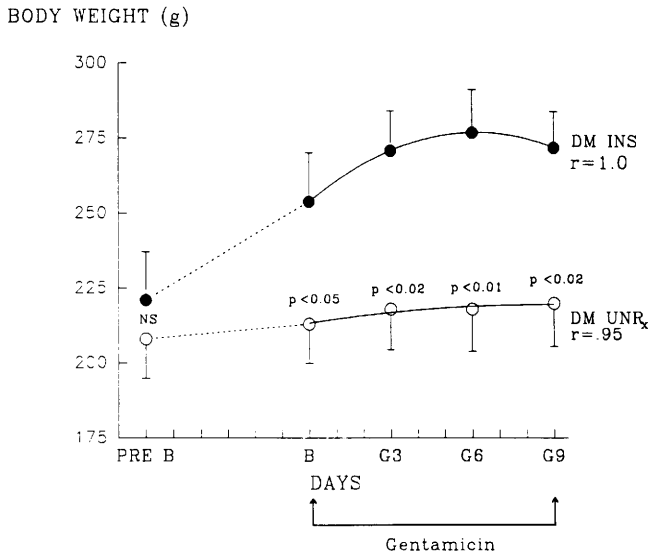

Figure 2. Weight changes recorded during the experiment. Lines drawn from regression analysis equation. The beneficial effects of insulin on body weight of the diabetic rat can be easily seen before the administration of G. All *P* values are DM-INS vs DM-UNRx. Note that body weights of female nondiabetic rats of similar age (7–8 months old) (not shown) are about 300–350 g.

Administration of Gentamicin. The DM-UNRx rats exhibited no signs of nephrotoxicity after 9 days of gentamicin administration. This dosage and duration of gentamicin treatment has been shown to reduce glomerular filtration rate (GFR) by 70%–95%, increase plasma creatinine concentration, and cause tubular necrosis in nondiabetic rats (1, 4). The average body weights of the DM-UNRx group increased slightly during this experimental phase ($P < 0.05$ vs B, Fig. 2). These animals maintained their high urine volume and glucose excretion, and muramidase was never identified in their urine (Table I). The tubular necrosis score was very low (median = 0, range from 0 to 1.5+), with only two animals showing single cell necrosis in sparse tubules (Table II).

In marked contrast, by Day G9 clear evidence of gentamicin-induced renal damage was present in the DM-INS rats. The urine volume, water intake, nonfasting plasma glucose and urinary glucose excretion remained stable during this phase. These values, however, were significantly lower than those observed in the untreated animals (Table I). Once correction of the diabetic state was accomplished, the rats gained approximately 5 g/day. This trend continued over the first 3 days of gentamicin administration, but the weight gain slowed to a mean of 2 g/day thereafter, and by Day G9 there was no further gain (Fig. 2). A reduction in food intake was observed on Day G6, but this change achieved significance only on Day G9 (Table I). Other evidence of nephrotoxicity appeared by Day G6, with an increase in plasma creatinine, and a reduction of Ccr that on Day G9 was 26% lower than at baseline ($P < 0.05$). Whereas both DM groups prior to commencing insulin therapy (PRE-B) had similar Ccr, by Day 9 of gentamicin treatment the Ccr in group DM-INS was 1.4 ml/min lower than that of the DM-UNRx group, a 45% difference ($P < 0.005$). Significant reductions in urine osmolality and urinary sodium excretion were also observed. In the DM-INS rats, urinary sodium concentration at baseline was similar to that of the DM-UNRx rats (28 ± 3 mEq/l). After gentamicin administration, however, it increased to 68 ± 8, 68 ± 7, 55 ± 6, and 42 ± 5 mEq/l on Day 1, 3, 6, and 9 of the study, respectively, whereas the corresponding values of the untreated rats remained unchanged (27 ± 3, 24 ± 1, 24 ± 2, 27 ± 2, and 28 ± 2 mEq/l,

Table I. Effect of Gentamicin Administration on Treated and Untreated Diabetic Rats

	Days of study	DM-INS (n = 7)	P	DM-UNR (n = 8)
Water intake (ml/day)	PRE B	108 ± 12	NS	112 ± 10
	B	46 ± 6†	<0.001	122 ± 10
	G3	54 ± 8	<0.001	129 ± 10
	G6	53 ± 6	<0.001	118 ± 12
	G9	56 ± 9	<0.001	111 ± 13
Food intake (g/day)	PRE B	28 ± 1	NS	27 ± 1
	B	26 ± 2	NS	28 ± 1
	G3	28 ± 2	NS	29 ± 1
	G6	24 ± 2	NS	26 ± 1
	G9	16 ± 2*	<0.001	27 ± 2
Urine volume (ml/day)	PRE B	89 ± 12	NS	94 ± 8
	B	29 ± 3†	<0.001	103 ± 8
	G3	33 ± 7	<0.001	105 ± 8
	G6	34 ± 4	<0.001	93 ± 8
	G9	38 ± 7	<0.001	94 ± 10
Plasma creatinine (mg/dl)	PRE B	.22 ± .01	NS	.22 ± .01
	B	.24 ± .01	NS	.22 ± .01
	G3	.24 ± .01	NS	.24 ± .01*
	G6	.28 ± .01*	<0.005	.21 ± .02
	G9	.39 ± .07*	<0.02	.19 ± .01*
Plasma glucose (mg/dl)	PRE B	542 ± 21	NS	594 ± 35
	B	131 ± 35†	<0.01	527 ± 24†
	G3	62 ± 3	<0.001	579 ± 16*
	G6	74 ± 18	<0.001	604 ± 19*
	G9	68 ± 11	<0.001	615 ± 37*
CCR (ml/min)	PRE B	2.5 ± 0.3	NS	2.4 ± 0.3
	B	2.3 ± 0.1	NS	2.5 ± 0.2
	G3	2.5 ± 0.2	NS	2.3 ± 0.2
	G6	2.0 ± 0.1	NS	2.5 ± 0.2
	G9	1.7 ± 0.3*	<0.005	3.1 ± 0.3*
Urine glucose (mg/day)	PRE B	8723 ± 882	NS	8472 ± 603
	B	450 ± 167†	<0.01	9614 ± 655
	G3	8.3 ± 2	<0.001	9465 ± 638
	G6	115 ± 66.5	<0.001	8202 ± 630*
	G9	279 ± 129	<0.001	8268 ± 892*
Urine osmolality (mOsm/kg H_2O)	PRE B	1156 ± 65	NS	1008 ± 88
	B	1169 ± 75	NS	995 ± 52
	G3	1128 ± 163	NS	944 ± 58*
	G6	942 ± 57	NS	958 ± 52
	G9	641 ± 72*	<0.005	956 ± 58*
Urine sodium (mEq/day)	PRE B	2.3 ± 0.1	NS	2.4 ± 0.2
	B	1.9 ± 0.1	<0.02	2.4 ± 0.2
	G3	2.1 ± 0.3	NS	2.5 ± 0.1
	G6	1.8 ± 0.2	NS	2.3 ± 0.1
	G9	1.4 ± 0.2*	<0.005	2.6 ± 0.2
Urine protein (mg/day)	PRE B	35 ± 16	NS	26 ± 5
	B	10 ± 4	NS	22 ± 6
	G3	10 ± 4	NS	22 ± 11
	G6	13 ± 4	NS	15 ± 4
	G9	24 ± 9	NS	18 ± 3

Note. PRE B: Day −7; B: average of Day −2 and −1 (see Fig. 1 for details); CCR: 24-hr endogenous creatinine clearance. NS: $P > 0.05$; †$P < 0.05$ vs PRE B; *$P < 0.05$ vs B.

respectively ($P < 0.001$ by repeated measure ANOVA). The increase in the urinary sodium concentration supports the presence of acute tubular injury in the DM-INS group, rather than that of a prerenal mechanism. Urinary muramidase (data not shown) was detected for the first time on Day G6, and by Day G9 its rate of excretion was 279 ± 113 μg per day. The tubular necrosis score was moderately high (median = 2, range from 1+ to 2.5+), and this value was statistically different from that of the DM-UNR rats ($P < 0.001$). In two rats there was single cell necrosis in sparse tubules (score of 1.0); in two animals the ne-

Table II. Pathology Scores of DM-UNR and DM-INS Groups After Gentamicin Administration

	DM-UNR (n = 8) (0–4)*		DM-INS (n = 7) (0–4)
	1.5		2.5
	0		1.0
	0		2.5
	0		2.0
	0		2.5
	0		1.0
	0.25		2.0
	0		
Median	0	$P < 0.001$†	2.0

* The arbitrary scale (range 0–4) used for the pathology assessment was described in Materials and Methods. The individual scores are the average of the evaluation of several coded slides.
† Mann-Whitney rank sum test.

crosis extended to other cells of the same tubule (score of 2.0); and in three rats, various tubules were affected (score of 2.5) (Table II).

The diabetic rats, as expected, had a greater urinary excretion of protein than that of age-matched nondiabetic animals (1, 4). However, no differences in the degree of proteinuria were detected between the two groups of diabetic rats nor within each group (By ANOVA) throughout the experiment (Table I). In contrast, a separate group of nondiabetic rats (n = 6) studied in our laboratory with a similar protocol (but not injected with insulin) increased their normal urine protein excretion 10-fold following gentamicin (baseline 4 ± 0.7 mg/day; Day 9 47 ± 13 mg/day; $P < 0.01$). These animals, as previously reported (1–5), developed gentamicin-induced acute renal failure (baseline Ccr 1.5 ± 0.2 ml/min Day 9; Ccr 0.4 ± 0.2 ml/min day; $P < 0.025$).

The kidney weight of DM-INS rats was 1.31 ± 0.1 g, a value not statistically different from that of the DM-UNR animals (1.41 ± 0.1 g). Likewise, no difference between groups was found in the water content of the kidney cortex (data not shown). The gentamicin content of the kidney cortex was 652 ± 37 μg/g of wet tissue in the DM-INS group, whereas the untreated diabetic rats exhibited a significantly lower value, 418 ± 48 μg/g of wet tissue ($P < 0.005$).

Discussion

In the present experiments we were able to demonstrate that when rats with streptozotocin-induced DM had their plasma glucose levels normalized by insulin, they showed clear evidence of decreased renal function and proximal tubular necrosis in response to gentamicin administration. This is in marked contrast to the observations made in this and other studies from our (1, 4), and other laboratories (2, 3) indicating resistance to the nephrotoxic effects of gentamicin administration in DM rats not receiving insulin.

As compared with nondiabetic rats (1, 4), the appearance of signs of nephrotoxicity was delayed in the DM-INS group, commencing by Day 6 of gentamicin administration. Furthermore, the magnitude of the renal injury, although statistically significant, was clearly less in the present study than previously reported for nondiabetic animals. This is not surprising, and suggests that the factors responsible for the protection were partially operative despite short-term normalization of nonfasting plasma glucose and glycosuria by insulin. All animals studied here, as in our previous studies (1, 4), had been diabetic for 6 months, whereas the group treated with insulin had their diabetic state corrected for only 2 weeks. Thus, it is conceivable that some metabolic aspects of diabetes were not normalized by insulin within the time frame of our experiments. For example, one week of insulin treatment successfully returns elevated plasma glucose, urinary volume, the protein/kidney weight ratio, and cortical and outer medullary Na,K-ATPase activity to control values, whereas regression of renal hypertrophy and reversal of absolute total renal Na,K-ATPase activity to normal levels requires at least three weeks of insulin therapy (11). Therefore, it is likely that the dose and duration of insulin administration used in the current study might not have been sufficient to reverse all the metabolic abnormalities associated with the diabetic state.

Although the findings reported for the first time in this study are straightforward, their interpretation is less clear. The possible roles of streptozotocin, insulin, solute diuresis, hyperglycemia, proteinuria, gentamicin pharmacokinetics, and some metabolic abnormalities of diabetes need consideration.

The possibility that streptozotocin might be involved in the protection observed in diabetic rats from gentamicin nephrotoxicity should be examined. Although under specific circumstances (high, repeated dosage) streptozotocin is known to be toxic to the proximal renal tubule (12, 13), a more recent study by Evan *et al.* (14) has failed to demonstrate any morphologic damage of this nephron segment after administration of a diabetogenic dose of this chemotherapeutic agent to Sprague-Dawley rats. Furthermore, when gentamicin was given to streptozotocin injected rats that had spontaneously lost their diabetic state, extensive tubular necrosis occurred as in the non-streptozotocin-injected animals treated with gentamicin (6).

Insulin has several known effects upon renal proximal tubular cells (15, 16), but to the best of our knowledge, there are no reports of any interaction between insulin and aminoglycoside transport. Therefore, it is

unlikely that the loss of the protection against gentamicin in the diabetic animals could be directly related to the hormone, rather, it appears that is the consequence of the correction of the diabetic state by insulin.

In previous studies, we demonstrated that solute diuresis induced by glycosuria in diabetic or in nondiabetic rats (phlorizin model) could not explain the protection against gentamicin nephrotoxicity (1, 5). Animals without glycosuria (nondiabetic rats) developed nephrotoxicity in response to gentamicin like rats with phlorizin-induced glycosuria. The renal cortical concentration of gentamicin in these groups was not different from each other or from that of diabetic rats with mild glycosuria that only developed minimal renal abnormalities after gentamicin (5).

Other investigators have examined the effect of osmotic diuresis in gentamicin nephrotoxicity (3, 17–19). The induction of an osmotic diuresis with the oral administration of isosorbide did not protect rats from gentamicin toxicity nor decrease its renal cortical accumulation (3, 17). In these studies, the magnitude of the solute diuresis achieved, although not reported in detail, was approximate to that observed in our rats with mild untreated diabetes, phlorizin-induced glycosuria (5) or that of the animals in the present study. Newman *et al.* (17) reported a urine volume of about 60–70 ml/day in their rats, and the dose of isosorbide used by Elliott *et al.* (3) was calculated on a molar basis to approximate the urinary glucose excreted by their diabetic rats (about 8–9 g/day). Prolonged salt diuresis induced either by DOCA-oral saline or furosemide-infused saline (urine volume, 70–90 and 50–70 ml/day, respectively) failed to afford functional protection, and actually increased the degree of morphologic damage induced by gentamicin (18). Likewise, oral salt loading for a shorter time also failed to alter the course of gentamicin nephrotoxicity in the rat (19). Furthermore, it is conceivable, that marked hyperglycemia and a high level of solute diuresis (glycosuria greater than 8 g/day) may alter the pharmacokinetics and/or tubular transport of gentamicin into the renal cortex and thus, minimize toxicity. The relationship between the level of nonfasting plasma glucose and glycosuria before the administration of gentamicin, and the change of GFR at the end of each study is shown in Figure 3. For this plot, data from the present and previous studies (1, 4, 5) were used. It is readily apparent that the animals with lesser degree of glycosuria exhibited greater decreases in Ccr, than those animals with marked hyperglycemia and glycosuria, despite receiving the same gentamicin dosage, suggesting a possible protective role for marked hyperglycemia and a high level of solute diuresis.

Consideration should be given also to the possibility that hyperglycemia itself may be in part related to

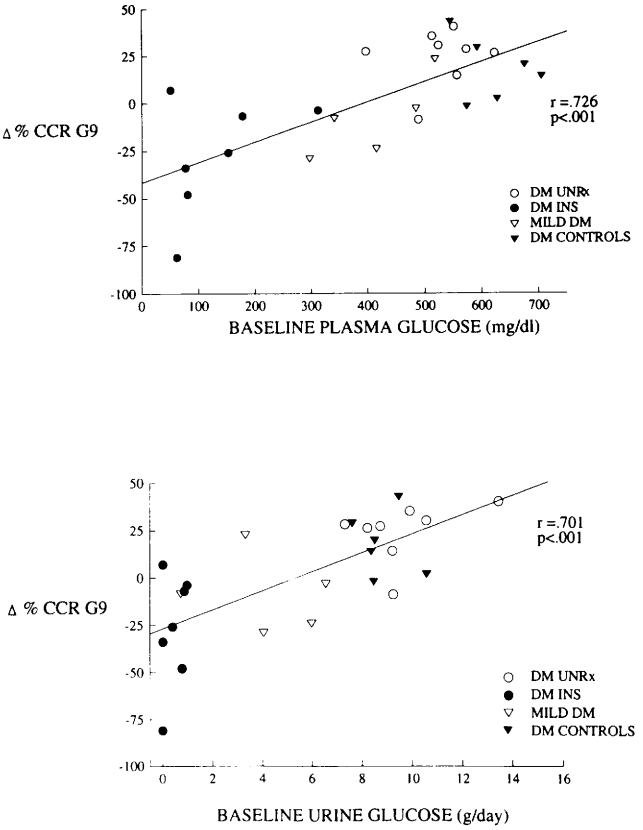

Figure 3. Relationship between the diabetic state (hyperglycemia and glycosuria) and decrease in renal function after 9 days of G administration (G9). The vertical axis represents the percent change of Ccr at Day G9 from baseline. The horizontal axis represents baseline plasma glucose (top) and urine glucose excretion (bottom). Four groups of animals are illustrated: DM-INS (●) (n = 7) and DM-UNRx (○) (n = 8), as defined in the present study. The data of the other groups shown (▽, ▼) were taken from published work from this laboratory (4, 5): MILD-DM (▽) (n = 5), a group of rats with diabetes induced either by administering subdiabetogenic doses of streptozotocin or subtherapeutic doses of insulin; these rats had mild to moderate glycosuria; DM-CONTROL (▼) (n = 6), animals with diabetes mellitus of 6-months duration with marked glycosuria but without implanted miniosmotic pumps. Their degree of glycosuria and percent change of Ccr was similar to that of group DM-UNRx. All animals were subjected to the same protocol of G administration used in the current study.

the protective effect of diabetes. This is based on the fact that the rats that we have treated with phlorizin (5), and those given isosorbide by others (3, 17), both maneuvers associated with a failure of protection from gentamicin toxicity or decreasing cortical gentamicin, exhibit glycosuria without hyperglycemia. Because of the increasing evidence that hyperglycemia may induce cellular injury in diabetic tissues, probably through peroxidation damage of membrane lipids (20, 21), this possibility should be taken into consideration. Furthermore, free-radical-mediated lipid peroxidation has been identified in the renal cortex of rats treated with gentamicin (22). Although controversy exists about the causative role of this process in the pathogenesis of the renal cortical cellular events leading to

aminoglycoside renal damage (22, 23), it should be noted that lipid peroxidation was demonstrated in the renal cortex when massive doses of gentamicin (200–400 mg/kg/day for 6 days) were given to diabetic rats (24). Unfortunately, studies to separate the effects of insulin administration from those of correction of hyperglycemia in the diabetic state (such as insulin-glucose clamp studies) were not performed in our animals.

It has been suggested that the degree of proteinuria may play a critical role in the renal cortical uptake of gentamicin and subsequent nephrotoxicity (25). It is well established that rats with streptozotocin-induced DM exhibit a significant proteinuria compared with nondiabetic animals (26, 27), and that protein excretion increases in nondiabetic rats following the administration of gentamicin (27–29). In 6-hr perfusion studies *in vivo,* Pattyn *et al.* (25) demonstrated that the renal cortical uptake of gentamicin was reduced in diabetic rats with modest proteinuria in comparison to control animals. It is thus conceivable that insulin treatment might decrease the proteinuria in the diabetic rats and therefore enhance gentamicin uptake and toxicity. The fact that insulin therapy in our animals did not change proteinuria following gentamicin administration suggests that proteinuria in itself probably cannot explain the protection afforded by diabetes and that other factors may be involved.

Abnormalities in the renal handling of aminoglycosides can be seen in association with untreated diabetes mellitus. In the untreated DM rat, gentamicin has a lower half-life, a higher plasma clearance, and, for any given dose tested, a substantially lower accumulation of gentamicin in the kidney cortex than that observed in the nondiabetic animals (30). Isosorbide did not influence serum gentamicin or its urinary excretion in the study of Newsman *et al.* (17). Unfortunately, the pharmacokinetics of gentamicin after insulin therapy were not evaluated in the present study.

Why untreated diabetic rats accumulate less gentamicin in their kidney cortex remains unclear (1, 4). As shown in microperfusion studies, high levels of glucose in the lumen of the proximal tubule do not interfere with gentamicin tubular uptake (31). Moreover, when phlorizin, a specific competitor of the glucose carrier, is given there was no decrease in cortical gentamicin or attenuation of acute renal failure induced by the aminoglycoside (5), nor decrease in the gentamicin uptake by brush border membrane vesicles (32). These data taken as a whole, suggest decreased binding and transport of gentamicin in diabetic rats (33). A recent study (34), however, reported no difference between the netilmicin or gentamicin binding constants of renal brush border and basolateral membrane vesicles from diabetic and nondiabetic rats, suggesting the existence of a postbinding defect in diabetic animals.

Elliott *et al.* (3) observed that the protection afforded in the rat by the diabetic state against gentamicin-induced nephrotoxicity resembled the resistance against gentamicin exhibited by rats recovering from mercuric chloride- (35), and potassium dichromate-induced acute renal failure (36). These authors showed a decrease in PAH uptake by cells harvested from the kidneys of diabetic animals before the administration of gentamicin, and suggested that a proximal tubule cell defect secondary to diabetes could be responsible for this phenomenon. More recently, Hazen-Martin *et al.* (37) were able to identify abnormalities in human kidney proximal tubule cells cultured in high glucose growth medium. Reduced dome formation coupled with alterations in electrical properties and in the ultrastructural characteristics of cellular organization were detected. This observation suggested that the paracellular, and perhaps the transcellular, routes of transport may be affected by high glucose levels. Finally, an increase in the activity of ouabain sensitive Na,K-ATPase in the proximal tubule has been demonstrated in association with the induction of diabetes mellitus (38). On the other hand, Cronin *et al.* (2) reported lower values at baseline and no changes in the activity of Na,K-ATPase in the renal cortex of the protected diabetic rats after 7 days of gentamicin administration.

Early biochemical cellular changes and a subsequent reduction of GFR characterize the natural history of experimentally-induced gentamicin ARF. The experimental and clinical evidence supporting the primary cellular changes, such as lysosomal phospholipidosis, labilization of lysosomes, and impaired mitochondrial respiration has accumulated rapidly (39–42). It is not yet clear, however, how these cellular events ultimately lead to the reduction in GFR and appearance of ARF.

Cell membrane phospholipids are considered the putative receptors for gentamicin (40, 41, 43), and low phospholipid content of the kidney cortex is a feature of diabetic rats (33). However, neither changes in the composition of nor abnormal *in vitro* binding of netilmicin by brush border or basolateral vesicles from renal proximal tubular cell membranes of diabetic rats have been demonstrated (34).

In summary, the protection observed in the diabetic rat against gentamicin nephrotoxicity appears due to the presence of the diabetic state and is reversed by the administration of insulin. The mechanisms responsible for this phenomena, and particularly the role of marked hyperglycemia and possibly high glycosuria, however, have yet to be established.

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