Strain differences in urinary factors that promote calcium oxalate crystal formation in kidney in ethylene glycol treated rats

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Running head: Strain differences in toxicity of ethylene glycol in kidney

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Abstract

Ethylene glycol (EG) induced hyperoxaluria is the most commonly employed experimental regimen as an animal model of calcium oxalate (CaOx) stone formation. The variant sensitivity to CaOx among different rat strains has not been fully explored, although the Wistar rat is known to accumulate more CaOx in kidney tissue after low-dose EG exposure than in the F344 rats. Supersaturation of CaOx in tubular fluid contributes to the amount of CaOx crystal formation in the kidney. We hypothesized that the urinary supersaturation of calcium oxalate in Wistar rats is higher than that of F344 rats, thereby allowing for greater CaOx crystal deposition in the Wistar rat. Age-matched male Wistar and F344 rats were treated with 0.75% EG or drinking water for 8 wk. 24-h urine was collected at 0, 2, 4, 6 and 8 wk for analysis of key electrolytes to calculate the CaOx supersaturation. Plasma oxalate level was also measured. Our data confirmed the different sensitivity to renal toxicity from EG between the two rat strains (Wistar > F344). After EG treatment, the plasma oxalate level and urine oxalate excretion were markedly greater in the Wistar rats than in the F344 rats, while urine calcium was slightly decreased in Wistars. Thus, the CaOx supersaturation in urine of Wistar rats was higher, which led to a greater crystal deposition in kidney in Wistar rats. These studies suggest that during EG treatment, changes in urine electrolytes and in calcium oxalate supersaturation occur to a greater extent in the Wistar rat, in agreement with its greater sensitivity to EG toxicity.

Keywords: Kidney stones; Animal model; Calcium oxalate crystals; Ethylene glycol poisoning
Introduction

Kidney stone disease is a common health problem; in the United States, it affects about 12% men and 5% women at some point in their lives (7). Although in most cases stones cause discomfort and inconvenience without significant risk to health, progressive loss of renal function can also occur with massive stone formation (46). Among the various stone compositions, calcium oxalate compromises the most prevalent component in kidney stones and hyperoxaluria is the most common metabolic abnormality in patients with kidney stone disease.

To better understand this disease, many experimental models have been developed to demonstrate calcium oxalate stone formation in animal kidneys (26). In the most common model, ethylene glycol (EG) has generally been employed as a oxalate precursor to induce hyperoxaluria, in an attempt to form calcium oxalate crystal deposition in the kidneys of rats (10, 26, 28).

A contributor to stone formation is calcium oxalate supersaturation (CaOx SS) in urine, which is the chemical driving force for crystallization. Urine is almost always supersaturated with respect to calcium oxalate. Animals develop hyperoxaluria, which elevates the level of CaOx SS in urine, thus promoting the nucleation and aggregation of microcrystals into larger crystals and eventually into clinically significant stones.

The toxicity of chronic EG exposure has been evaluated in a variety of species. Though the kidney is identified as the primary target organ, a considerable range of sensitivity to EG exposure has been observed across species, strains and sexes. Cruzan et al (9) have reported that there is a major strain difference in sensitivity to EG-induced renal toxicity in male F344 and Wistar rats treated with EG (150~1000 mg/kg BW) for 16 weeks. Wistar rats are more sensitive to renal toxicity than F344 and the
renal toxicity in both rat strains is closely related to the accumulation of CaOx crystals in kidney tissue (9). The mechanism for this strain-related difference is still under investigation. Previously, we have demonstrated that the CaOx crystal but not the oxalate ion produces cytotoxicity in renal tubule cells (18, 19), and that the sensitivities to CaOx crystals are the same in the proximal tubule cells from the two rat strains (18). Thus we hypothesize that the strain-related difference in the toxicity of CaOx crystals is related to the different supersaturation state of the calcium oxalate in urine, allowing for greater CaOx crystal formation in the Wistar rat. To investigate our hypothesis, we treated age-matched male Wistar and F344 rats with EG for as long as 8 weeks and observed the changes in the urinary variables and supersaturation state with respect to calcium oxalate in urine in both rat strains. A goal of the study was to provide an insight into the rodent model that can best mimic the stone disease in humans and to assist in human health assessments of ethylene glycol toxicity.

Materials and Methods

Animal Protocol

Male Wistar and F344 rats (Harlan, Indianapolis, Indiana) at 11 weeks of age were randomly divided into four control and treated groups per strain. The animals in these groups were treated for 2, 4, 6 or 8 weeks, with 4 or 5 per treatment group. Control rats received normal drinking water, while treated animals were given 0.75% (vol/vol) EG as their water source to induce a chronic mild hyperoxaluria and calcium oxalate deposition in kidneys (16, 17, 32). The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark) and were allowed free access to food (normal rat chow). Water and fluid consumption was recorded daily. Rats were weighed before and during the treatment to assess growth. Urine was collected before and during treatment to analyze urinary electrolytes. At least 4 control rats and 4 experimental rats from each strain were
examined at each time point. In the 8 week treatment groups, additional urine samples were collected from all animals at the 2, 4 and 6 week time points. The animal protocols were approved by the Institutional Animal Care Committee (Louisiana State University Health Sciences Center-Shreveport) and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Urine Collection and Analysis

The rats were placed in metabolic chambers to obtain a 24-hour urine collection using ice-jacketed 50 mL tubes to minimize bacterial growth. After determining urinary volume and pH, urine samples were settled for 30 minutes to remove debris. Then the urine was aliquoted for various assays, including calcium, oxalate, citrate, magnesium and creatinine, as described below. The aliquots for calcium and oxalate assay were acidified to dissolve the CaOx crystals before further analysis as described by Hodgkinson et al (22, 36, 47).

Urinary Electrolytes and Constituents

Calcium (Ca\(^{2+}\)) concentration in urine was measured by an arzenazo III method as described by Leary et al (30); oxalate (Ox) was measured with an oxalate assay kit from Trinity Biotech (Wicklow, Ireland), which employs the oxalate oxidase method (22, 36, 47); magnesium (Mg\(^{2+}\)) by an isocitrate dehydrogenase enzymatic method as described by Stone et al (40); citrate (Cit) by a citrate lyase enzymatic method as described by Hosking et al (23); creatinine was measured by an creatinine assay kit from Cayman Chemicals (Ann Arbor, Michigan).

Calcium Oxalate Supersaturation (CaOx SS)

The CaOx SS can be assessed by a computerized iterative approximation with a program named EQUIL 2, developed by Finlayson et al (42, 45). However, a large number of variables have to be
analyzed for these calculations, particularly in animal models when the urine volume is limited. Here the ion-activity product was determined by an equation validated by Tiselius et al (43) specifically for rat urine, which gave a simple estimation of CaOx SS. The ion-activity product of calcium oxalate (APCaOx) in rat (AP(CaOx)indexRAT), as an index of CaOx SS, was calculated as follows:

\[
AP = \frac{4067 \times Ca^{0.93} \times Ox^{0.96}}{(Citrate + 0.015)^{0.60} \times Mg^{0.55} \times Volume^{0.99}}
\]

This equation was designed specifically for a 24-h collection period and the total excretions of Ca\(^{2+}\), Ox, Mg\(^{2+}\) and citrate were expressed in millimoles. To compare the calculated AP(CaOx)indexRAT with the CaOx SS from EQUIL 2, an aliquot of selected urine samples were submitted to Omega Diagnostics for electrolyte testing. EQUIL 2 was then used to calculate CaOx SS.

Plasma Oxalate Concentration

At the end of 2 weeks treatment, 5 rats from each treatment group were anesthetized with sodium pentobarbital and blood was drawn from the inferior vena cava into heparinized tubes. The plasma oxalate (Ox) was measured with an oxalate assay kit from Trinity Biotech (Wicklow, Ireland) as described by Lieske et al (29), with modifications for measuring the low oxalate levels in plasma.

Kidney Tissue Processing

At 2, 4, 6 and 8 weeks, the animals (4 or 5 per group) were anesthetized with sodium pentobarbital and the kidneys were rapidly removed. Half was fixed in neutrally buffered 10% formalin for histological processing and the remaining was homogenized in ice-cold Tris–HCl buffer (0.1 M, pH 7.4) and was frozen at -20 °C for future analysis.
Histopathological Studies

A slice of kidney tissue was fixed in 10% neutral buffered formalin. Tissues were washed and dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 4 μm thickness, and stained with haematoxylin and eosin. The sections were analyzed by light microscopy as well as polarizing microscopy for CaOx crystal deposition and other histopathological changes. The presence of CaOx crystals in kidney tissue was scored as 0 to ++++ (9).

Statistical Analysis

Differences between groups were assessed by one way analysis of variance (ANOVA) with student-Newman-Keuls test to compare differences among treatment groups, using the SAS software package for Windows. The level of significance at P<0.05 was considered significant.

Results

*EG treatment does not affect body weight gain or fluid consumption.*

Body weight increased gradually in all of the groups. The EG treatment did not affect body weight in either rat strain (student-Newman-Keuls test, P>0.05) (Table 1). Compared to the age-matched Wistar rats, F344 rats had lower body weights in both control and treated groups (P<0.05). The body weights increased similarly in the control and treated rats in both strains throughout the time period. The kidney/body weight ratio did not show a difference between the strains or treatment groups at all the time points (Table 1). In the 8 week group, one of the four treated Wistar rats showed enlarged, fibrotic
kidneys such that the kidney/body weight ratio was 0.0089. Fluid consumption, adjusted by the body weight, was not different at any time point between the two rat strains (Table 1). The animals in the treated groups from both strains consumed similar amounts of EG and did not show a significantly higher consumption than controls. The daily dosage of EG consumed by each rat was calculated from the fluid consumption, the EG concentration (0.75% vol/vol), and the body weight. Rats in both strains were exposed to similar doses of EG throughout the time course (F344 887±102 vs Wistar 997±199 mg/kg BW/Day). The 24-hour urine volume was higher in Wistar than in F344 rats, but after being adjusted by the body weights, the urine volume was not different between strains, nor between control and treated rats. After adjusted by body weight, creatinine excretion remained relatively unchanged (data not shown), either by time or by EG treatment in both strains. The lack of any differences is important, since subsequent values of urinary excretion by individual rats were normalized by creatinine excretion.

**Histology of EG-induced nephrotoxicity**

With examination of tissues by polarizing microscopy, treated Wistar rats showed evidence of crystal deposition in the kidney and of an associated crystal-induced kidney tissue damage after 4 weeks EG treatment (Fig. 1). Crystal deposition in the kidney and associated kidney damage were observed in 3 of the 5 Wistar rats at 4 weeks, 2 of 4 at 6 weeks and 3 of 4 at 8 weeks (Table 2). The fixed kidney sections showed that the accumulation of birefringent CaOx crystals was located exclusively in the renal tubule lumen, although some microcrystals were also found under the basement membrane and in the interstitium. In severe cases, the crystals occluded the tubule lumen, particularly proximal tubules. The tubule lumens were dilated, with the epithelial lining damaged at variant degrees. No crystals and no
tissue damage were seen in control F344 or control Wistar rats at any time point, nor in treated F344 rats as long as 8 weeks treatment (Fig. 1 for the 4 week time point).

**Effect of EG treatment on urinary excretion of electrolytes and key constituents**

Urinary pH was not significantly affected by EG treatment at any time point in either rat strain, although it was slightly decreased at 8 weeks in both treated F344 (pH 6.14 vs 6.29) and Wistar rats (pH 6.10 vs 6.34) (Fig. 2). Urinary excretion of oxalate increased with time in EG-treated Wistar rats, but not in F344 rats (Fig. 3), with significant increases being observed in treated Wistar rats as early as 2 weeks. Total urine oxalate excretion adjusted by creatinine (mmol/g Cr. /day) increased from 0.3 on week 0 to 2.1 on week 2 in treated Wistar rats. Substantial increases in oxalate excretion were further seen in 4, 6 and 8 weeks, respectively. The urinary oxalate concentration (mmol/L of urine) showed the same trend (data not shown). Neither total oxalate excretion nor urine oxalate concentration was significantly increased in EG-treated F344 rats at any time period. Total urine calcium excretion was decreased in treated Wistar rats, particularly at 4 and 6 weeks (Fig. 4). Similar decreases in urine calcium concentration (mmol/L) were observed in EG-treated Wistar rats (data not shown). No significant differences were found in treated F344 rats compared to the controls in both urinary calcium concentration (data not shown) and total urinary calcium excretion (Fig. 4). Citrate and magnesium, as modulators of CaOx crystallization, are important factors in determining CaOx SS. However, no significant differences were demonstrated in urinary citrate and magnesium concentrations (Fig. 5&6), nor in the total excretions in urine (data not shown).
Supersaturation with respect to calcium oxalate after EG treatment

The CaOx SS can be assessed by a computer program named EQUIL 2, or by the equation proposed by Tiselius et al (43). In a preliminary analysis, the calculated AP(CaOx)indexRAT was observed to correlate with the CaOx SS obtained from EQUIL 2 (Fig. 7). The correlation coefficient for these calculations was 0.40, due to the higher AP(CaOx)indexRAT value and relatively low CaOx SS value from 4 treated Wistar rats, which showed markedly elevated urine oxalate concentrations. If the data from these 4 rats were excluded, the resulting correlation coefficient would be 0.86. This result agrees with the calculation of Tiselius et al (43) and indicates that the calculated AP(CaOx)indexRAT is a valid index for CaOx SS.

The CaOx SS as indicated by the calculated AP(CaOx)indexRAT did not show a difference between untreated rats from the two strains (Fig. 8). Similar to the increased trend of urinary oxalate excretion, the CaOx SS increased significantly in treated Wistar rats as early as 2 weeks, and further increased markedly compared to the control groups and to EG-treated F344 groups with longer treatment times. EG-treated F344 rats, however, also showed a significant increase in CaOx SS at both 2 and 4 weeks, but not at 6 and 8 weeks.

Plasma oxalate levels in EG treated Wistar and F344 rats

The oxalate levels in plasma were low in control groups in both rat strains, especially compared to those in humans (0.1 µmol/L vs 1.0 µmol/L) (Fig. 9) (29). The Wistar rats showed a marked increase in the oxalate level in plasma after 2 weeks EG treatment, and the increase was correlated with the oxalate level in urine in treated Wistar rats. The EG treatment did not significantly change the oxalate level in plasma in F344 rats.
Discussion

Among possible animal models for the study of stone disease, rats are the animals most commonly used (28). Oxalate metabolism in humans and rats is considered to be similar (26), and chronic mild hyperoxaluria can cause CaOx stone formation in both humans and rats (17, 27). Chronic low dose administration of EG to rats is often used to model stone formation (17, 27). The variant sensitivity to CaOx among different rat strains, however, is not widely known. Cruzan et al reported a strain-related difference in sensitivity to chronic EG exposure between two rat strains, Wistar and F344 rats (9). Wistar rats treated for 16 wk show markedly more CaOx crystal deposition and renal damage than do F344 rats at EG doses ≥500 mg/kg, and the degree of renal tissue damage is very closely related to the amount of CaOx crystals in kidney tissue in both strains. To investigate the mechanism for this strain difference, the current study examined the strain differences in urine constituents that control formation of oxalate crystals, i.e. CaOx supersaturation (CaOx SS), in relation to the time course for deposition of the oxalate crystals. Our data show that the CaOx SS was increased after EG treatment to a greater extent in the Wistar rats, thus explaining the greater CaOx crystal accumulation. CaOx crystals were deposited in the kidney of Wistar rats only at the time course when the CaOx SS reached the critical level as discussed below. Furthermore, in the F344 rats, CaOx crystals were not seen and there was only a small increase in the CaOx SS. This study has also confirmed that the strain difference in CaOx accumulation occurs with the EG drinking water model. Cruzan et al (9) conducted a toxicologic study for which EG administration in the diet is optimal, however, models of kidney stone formation have used the drinking water exposure. Our data show that rats treated with EG in drinking water at a dose of about 900 mg/kg BW/day have crystal deposition in kidneys of Wistar rats as soon as 4 wk, but not any crystals in F344 rats as long as 8 wk, thus confirming the strain difference with this model.
Finally this study has demonstrated that the mechanism for the increase in the CaOx SS was the marked increase in urinary oxalate excretion in Wistar rats, in as early as 2 wk, without any changes in other constituents that modulate CaOx formation. In contrast, F344 rats excreted only slightly more oxalate than control and much less oxalate than treated Wistar rats at all time points. Similar levels of urinary oxalate were seen in these two strains in the dietary study (9), while Sprague-Dawley rats treated with the drinking water regimen also had similar increases in urinary oxalate excretion as the Wistar rats (17). The oxalate levels in urine in both rat strains correlated with the presence or absence of changes in CaOx SS in urine and with the amount of crystal deposition in kidney.

CaOx crystallization is a kinetic phase change process, and the phase changes are driven by the urinary supersaturation with respect to CaOx. The CaOx SS can be estimated by an urinary ion equilibrium program originally verified by Finlayson (13) in 1977. Werness et al modified it into a computer-based program named EQUIL 2 (45). Although this program has been considered as the standard for estimation of CaOx SS, up to 14 variables have to be analyzed for these calculations, which is both expensive and technically demanding. An equation proposed by Tiselius et al (42) to calculate the APCaOx index has been accepted as a simple, but valid estimation of CaOx SS in urine. Parks et al have also reported that the composition of stones correlates with the APCaOx index values from the urine of the stone forming patients (35). Recently Tiselius has presented a modified APCaOx index for estimation of CaOx SS in rat urine (43), which only requires measurement of urinary calcium, oxalate, citrate, magnesium and urine volume. A constant of 4067 is employed to adjust the AP(CaOx)indexRAT to 1 at the equilibrium of CaOx saturation in urine. Therefore at APCaOx index values less than 1, crystals of CaOx will dissolve; at values greater than 1, crystals can aggregate and nucleate. In our preliminary analysis from age-matched Wistar and F344 rats treated with EG, the calculated
AP(CaOx)indexRAT was observed to correlate well with the CaOx SS obtained from EQUIL 2, except in a few samples at extremely high urine oxalate concentration, when the EQUIL 2 value seemed to be lower than expected. The AP(CaOx)indexRAT did not show a difference between the two rat strains before treatment, with values less than 1 suggesting little to no crystal nucleation. However, the AP(CaOx)indexRAT was significantly increased in both Wistar and F344 rats after 2 wk treatment with a greater increase in Wistar rats as expected from the higher oxalate levels in urine. CaOx crystals were not seen in treated Wistar rats until 4 wk, when the AP(CaOx)indexRAT value was above 2.0. The increase of AP(CaOx)indexRAT in treated F344 rats was only to a value around 1.2, and no crystals were observed in the kidneys. In F344 rats, a low oxalate and relatively normal calcium excretion may explain the small increase in CaOx SS, but lack of crystal deposition. Thus, the strain differences in CaOx SS after EG treatment can explain why CaOx crystals form and accumulate to a higher degree in the Wistar than in the F344 rats.

Hyperoxaluria, defined as an increased oxalate excretion exceeding the normal range, is an important risk factor for the pathogenesis of calcium oxalate stone disease. Increased urinary oxalate may promote the supersaturation with respect to calcium oxalate. In the study by Cruzan et al (9), urinary oxalate concentrations were increased in Wistar rats at 1 wk, compared to that in F344 rats. We observed similar increases in urine oxalate by 2 wk. However, in addition, the present studies in these rat strains have demonstrated that the primary determinant of crystal formation in the EG treated rat is the marked increase in oxalate levels in the urine. EG treated Wistar rats excreted markedly more oxalate than did F344 rats, and as discussed later, excreted less calcium. These factors led to the large increase in CaOx SS in the urine of Wistar rats, which in turn contributed to the formation and accumulation of
CaOx crystals in kidney. CaOx SS was somewhat elevated in EG-treated F344 rats, but apparently not enough to promote crystal formation and retention.

Hypercalciuria is found in up to 40% of stone-formers (7) and urinary calcium is another important risk factor in crystal formation. Bushinsky et al have established a genetic hypercalciuric rat model which shows an increased incidence of stone formation (4). However, hypercalciuria has less impact than hyperoxaluria on CaOx SS in urine. In humans, urine normally contains about 1-5 mmol/L calcium but only 0.1-0.5 mmol/L oxalate (37, 38), which means that changes in oxalate level should have a larger effect on CaOx SS and the subsequent crystallization compared to changes in urinary calcium. In this experimental regimen, hypercalciuria had a lesser effect on CaOx SS and crystal formation. The urinary calcium in F344 rats did not change throughout 8 wk EG treatment. In contrast, a 50% decrease in urine calcium was observed after 4 wk in treated Wistar rats. A similar decrease was also seen in Sprague-Dawley rats with same regimen (17). This decrease in calcium may have resulted from the increase in oxalate crystal retention in the kidney (oxalate trapping of the calcium), because it was only observed in rats (Wistar) with kidney crystals and not in F344 rats without crystals. Despite the decrease in urine calcium, CaOx SS was increased in Wistar rats because of the high oxalate excretion.

Magnesium and citrate are modulators of crystallization by combining with oxalate and calcium, respectively, thus reducing the supersaturation of calcium oxalate. Magnesium deficient diets may induce CaOx crystal deposition in rats, but excess magnesium supplementation does not affect CaOx SS and crystallization in hyperoxaluric rats (41). Citrate is an inhibitor of CaOx crystallization in patients with hypocitraturic CaOx nephrolithiasis. Hypocitraturia was seen in 16-63% of recurrent stone formers (34). Citrate in the urine chelates with calcium and forms a soluble complex, thus reducing calcium ion activity and CaOx SS (39). Citrate can also adhere to the surface of CaOx crystals to prevent crystal
nucleation and aggregation (6). Citrate supplementation is a general treatment in patients with kidney stones. In our study, however, neither magnesium nor citrate in urine was changed by EG treatment in either rat strain at any time point. This indicated that the strain-related difference in sensitivity to EG treatment was not associated with a different clearance of magnesium or citrate.

Although EG induced hyperoxaluria is the most commonly employed experimental regimen in animal models of kidney stone formation, the model has been criticized for possibly producing metabolic acidosis (2, 12). This concern generally arises from clinical observations in humans of EG overdoses (11, 15, 24, 25). After ingestion, EG can be absorbed and metabolized into glycolate and oxalate rapidly in the liver. Metabolic acidosis is then induced due to the accumulation of EG metabolites, principally glycolate in the body at high doses of EG (5, 14). In 2005, Green et al (17) demonstrated that EG consumption in a low dose rate regimen (0.75% vol/vol in drinking water) for 4 wk did not cause metabolic acidosis in Sprague-Dawley rats. Consistent with these studies, Wistar and F344 rats treated at the same doses showed no evidence of acidosis, except for a slight decrease in urine pH in treated Wistar and F344 rats after 8 wk. The excretion of glycolate or other acid metabolites was most likely responsible for this small decrease in urine pH. It is unlikely that the decreased urine pH results from renal dysfunction, since the renal function appeared unchanged in both strains as indicated by the lack of differences in creatinine excretion and in urine volume. Metabolic acidosis may also be manifested as decreased urinary citrate excretion (20) or enhanced urinary calcium excretion (3). In our study, no decrease was observed in urinary citrate in any treatment group and urinary calcium was either unchanged or decreased as in treated Wistar rats. Thus, this experimental regimen does not elicit significant metabolic acidosis in either rat strain, yet produces marked oxalate accumulation in only the Wistar rat.
Previous studies on the strain difference in crystal accumulation showed that there are no
differences in the binding and internalization of COM crystals by the proximal tubule cells (31). Instead,
our studies have shown that the strain difference occurs because of the underlying differences in urinary
factors that promote crystal formation, i.e. CaOx SS (after EG treatment, CaOx SS in Wistar is greater
than in F344). The greater CaOx SS in the EG treated Wistar rats results from the increased urinary
oxalate excretion. The reason for the greater urinary oxalate excretion in the Wistar rat is not known. In
Cruzan’s study (9), the oxalate levels in blood were slightly elevated in Wistar rats compared to F344
rats by 1 week at the EG dose of 1000 mg/kg. However, the blood oxalate levels in treated Wistar rats
were not elevated compared to Wistar rats dosed at 0 mg/kg (controls), indicating the absence of a true
increase. In contrast, a markedly increased oxalate level in plasma was observed in Wistar rats after 2
wk EG treatment in our study, both compared to the Wistar controls and to the treated F344 rats. The
lack of an increase in the Cruzan study may have resulted from the higher background blood oxalate in
their controls (possibly due to dietary differences between the studies) or from a difference in response
of plasma versus blood oxalate.

Possible explanations for the strain differences in oxalate levels in the urine and plasma after EG
treatment include a greater production of oxalate in the liver of the Wistar rats, or a lesser degree of
clearance of oxalate by the kidney. It is known that defects in several enzymes in oxalate metabolism
may cause hyperoxaluria (21, 44), although such genetic disorders are relatively rare in human and not
well known in rats. The metabolism of EG to glycolate in the liver in Wistar rats is probably not
different, because Cruzan et al (9) have reported in EG treated rats, that blood EG and glycolate levels
are similar in Wistar and F344 rats. The metabolism of glycolate (or glyoxylate) to oxalate, however,
may be different in the two strains, as a much higher oxalate level in plasma was observed in Wistar rats.
compared to that in F344 rats. The higher level in the plasma suggests there is also a higher delivery of
oxalate to the kidney, which could itself explain the higher urinary oxalate excretion in the Wistar rats.
Strain differences in the hepatic enzymes and activities that produce oxalate have not been characterized.

Another factor that could contribute to the strain difference could be the differences in renal
clearance of oxalate. A recent study by Corley et al (8) measured the oxalate clearance in both strains
after infusion of $^{14}$C-labeled oxalate and found that there is no difference in the total renal oxalate
clearance between Wistar and F344 rats. However, when normalized to body weight (which is higher in
Wistar rats), the oxalate clearance appeared lower in Wistar rats, which could result in more oxalate
retention in the kidney. Whether this clearance difference is a major factor in the strain difference is not
known. Further studies of the transport of oxalate by the proximal tubule cells of the two strains might
clarify this, because renal clearance would be controlled by reabsorptive and secretory transport of
oxalate. There are two kinds of transporters involved in oxalate excretion. SLC26A1 is a
sulfate/oxalate/bicarbonate exchanger found in the basolateral membrane of proximal tubule cells, and
SLC26A6 is the primary apical proximal tubule oxalate transporter (1, 33). The expression and activity
of the oxalate transporters in the two rat strains and whether these transporters are modulated by EG
treatment are not known. The data indicating a higher plasma oxalate in the Wistar rat would suggest
that the key strain difference may not be the renal handling of oxalate, but may result from differences in
hepatic production of oxalate.

In conclusion, we compared two rat strains, Wistar and F344 rats as animal models of
hyperoxaluria. Our results using a more commonly used EG treatment regimen confirmed the different
sensitivity to renal toxicity between the two rat strains. These studies have supported the hypothesis that
there is a higher CaOx SS in the EG treated Wistar rats, allowing for greater CaOx crystal formation and
deposition in the Wistar rats. A novel finding of this study is the observation that EG treated Wistar rats have higher oxalate levels in plasma. Further studies are needed to examine the hepatic oxalate formation and the expression and activity of oxalate transporters in proximal tubule in the two strains, which should provide a mechanistic explanation for the strain difference in oxalate crystal accumulation.
References


Table 1. Effect of EG treatment on body weight and renal function in F344 and Wistar rats treated orally with EG.

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<td>4</td>
<td>13.1 ± 5.2 ^a</td>
<td>10.9 ± 2.1 ^a</td>
</tr>
<tr>
<td>6</td>
<td>7.8 ± 0.8 ^a</td>
<td>9.6 ± 1.2 ^a</td>
</tr>
<tr>
<td>8</td>
<td>9.3 ± 1.4 ^a</td>
<td>10.1 ± 3.1 ^a</td>
</tr>
</tbody>
</table>

Rats treated with 0.75% EG or control (water) for 2, 4, 6 and 8 weeks. Data are represented as mean ± SD (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). ^a. Significant compared between strains at same treatment and time course (p < 0.05). ^b. n=4 or 5.
Table 2. COM crystal accumulation in kidney tissue in EG treated Wistar rats.

<table>
<thead>
<tr>
<th>Weeks of treatment</th>
<th>Degree of Crystal Deposit in Kidney</th>
</tr>
</thead>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

Rats treated with 0.75% EG or control (water) for 2, 4, 6 and 8 weeks. Data are represented as numbers of rats in each group (totally 4 or 5 per group). 0 indicates that no crystal deposition was observed; +~ + + as few to mild crystal deposits; + + + + as a large amount of crystal deposits.
Figure Legends:

**Fig. 1** Crystal accumulation in kidney tissue in EG treated Wistar rats, but not in kidney tissue in EG treated F344 rats. A. F344 rats in control group. B. F344 rats treated with 0.75% EG for 4 weeks, completely crystal-free in kidney. C. Polarized light optical photomicrograph of field (B) at same magnification. D. Wistar rats in control group. E. Wistar rats treated with 0.75% EG for 4 weeks, with large amount of crystals accumulated, especially within the tubular lumen. F. Polarized light optical photomicrograph of field (E) at same magnification. Retained crystals exhibit strong birefringence (magnification, x200).

**Fig. 2** Urine pH in F344 and Wistar rats treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean ± SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test did not show any significant differences between strains at any time point or between control and treated rats at any time point (P<0.05).

**Fig. 3** EG treatment increased urinary oxalate excretion in Wistar rats, but not in F344 rats. Rats were treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean ± SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test. # Significant compared to control group at same treatment time (p < 0.05). $ Significant compared between strains at same treatment time (p < 0.05).

**Fig. 4** EG treatment decreased total calcium excretion in urine in Wistar rats, but not in F344 rats. Rats were treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean ± SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test. # Significant
compared to control group at same treatment time (p < 0.05). $ Significant compared between strains at same treatment time (p < 0.05).

**Fig. 5** Citrate concentration in urine is not affected by EG treatment in F344 and Wistar rats. Rats were treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean + SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test showed no differences.

**Fig. 6** Magnesium concentration in urine is not affected by EG treatment in F344 and Wistar rats. Rats were treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean + SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test showed no differences.

**Fig. 7** Relationship between the CaOx SS and AP(CaOx)indexRAT in 24-h urine samples. The CaOx SS was derived from calculations with EQUIL 2 and the AP(CaOx)indexRAT according to the formula described in Methods section.

**Fig. 8** Supersaturation with respect to CaOx in urine in F344 and Wistar rats treated with 0.75% EG or water for 2, 4, 6 and 8 weeks. Data are represented as mean + SEM (n = 16 or 17 at wk 0, n = 8 or 9 at wk 2, 4 & 6, n = 4 at wk 8). Student-Newman-Keuls test. # Significant compared to control group at same treatment time (p < 0.05). $ Significant compared between strains at same treatment time (p < 0.05).

**Fig. 9** Plasma oxalate concentrations in F344 and Wistar rats treated with 0.75% EG or water for 2 weeks. Data are represented as mean + SEM (n = 5). Student-Newman-Keuls test. # Significant compared to control group at same treatment time (p < 0.05). $ Significant compared between strains at same treatment time (p < 0.05).
Fig. 2.

The bar graph shows the urinary pH levels over a period of treatment weeks for different groups:

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated

The x-axis represents the treatment weeks, and the y-axis represents urinary pH levels from 5.0 to 7.0.
Fig. 3.

Total Oxalate Excretion in Urine (mmol Ox / g Cr./ Day)

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated

Legend:
- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated

Treatment (Weeks)
Fig. 4

Urinary Calcium Excretion in Urine (mmol Ca / g Cr./ Day)

Treatment (Weeks)

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated
Fig. 5

Citrate Concentration in Urine (mg/dL) vs. Treatment (Weeks)

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated

The graph illustrates the citrate concentration in urine over different treatment weeks for two types of rats, F344 and Wistar, with and without treatment. The concentration is measured in mg/dL and is shown for various treatment durations (0, 2, 4, 6, 8 weeks). The data suggests a trend of increased citrate concentration with longer treatment periods for both control and treated groups, although the specific concentrations are not detailed in the image.
Fig. 6

Magnesium Concentration in Urine (mmol/L) vs. Treatment (Weeks)

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated
Fig. 8

![Graph showing the AP(CaOx)indexRAT over treatment (weeks). The X-axis represents treatment (weeks) from 0 to 8, and the Y-axis represents the AP(CaOx)indexRAT ranging from 0 to 6. The graph compares F344 Control, F344 Treated, Wistar Control, and Wistar Treated groups.](image)

Key:
- Open bars: F344 Control
- Gray bars: F344 Treated
- Light gray bars: Wistar Control
- Dark gray bars: Wistar Treated

Significance levels:
- # indicates a significant difference from the control group.
- $ indicates a significant difference from the treated group.
- $# indicates a significant difference from both control and treated groups.

Note: The exact values and statistical significance are not provided in the image.
Fig. 9

Plasma Oxalate Concentration (µmol/L)

- F344 Control
- F344 Treated
- Wistar Control
- Wistar Treated

Symbols:
- $\#$: Statistical significance