Uremic Levels of Oxalic Acid Suppress Replication and Migration of Human Endothelial Cells

Richard I. Levin, Philip W. Kantoff, and Eric A. Jaffe

Patients with chronic renal failure who undergo hemodialysis experience accelerated atherosclerosis and premature death. Since the end-metabolite, oxalic acid, accumulates in plasma in proportion to the severity of renal failure, we studied whether sodium oxalate (0 to 300 μM) is an endothelial toxin and, therefore, might enhance atherogenesis. Exposure to uremic levels of oxalate (>30 μM) for 9 to 28 days depressed endothelial cell replication by 33% to 84% (mean±SD, 54%±15.7%, n=17 experiments, p=0.002). In contrast, replication of fibroblasts exposed to 200 μM oxalate for 45 days was not inhibited. The inhibitory effect of oxalate on endothelial cell replication was both dose- and time-dependent (both p<0.0001) and was first detected 3 to 7 days after the initial exposure to oxalate. Further, the inhibitory effect was fully reversible upon removal of oxalate, but only if exposure was limited to 5 days or less. Sodium salts of other carboxylic acids (citric, succinic, glyoxylic, and malonic; 200 μM) as well as HCl (200 μM) did not suppress endothelial cell replication. Oxalate also inhibited endothelial cell migration but had no effect on basal, thrombin-induced, or arachidonate-induced prostacyclin production by endothelial cells. Exposure of endothelial cells to sodium oxalate (200 μM) for as little as 24 hours—a time period sufficient to induce delayed, transient inhibition of replication not detectable until approximately 1 week after exposure—inhibited incorporation of 3H-leucine into protein by 40% (p=0.009). We conclude that sodium oxalate acts as an uremic toxin, inhibiting endothelial cell replication and migration, functions which may be important for constitutive inhibition of atherosclerosis. (Arteriosclerosis 10:198–207, March/April 1990)

The incidence of both atherosclerosis and its complications is elevated in patients with uremia who are maintained on chronic dialysis.1-7 Further, this morbidity occurs at a much younger age than in the general population,4-8 suggesting that atherosclerosis is both more prevalent and accelerated in patients with chronic renal failure. The mechanism of accelerated atherosclerosis in uremia is probably complex; hypertension,6-9 abnormal carbohydrate metabolism,10,11 a prothrombotic state accompanied by decreased fibrinolysis,12,13 and abnormal factor VIII,14-16 abnormal lipoprotein patterns,17-25 polycystic, glyoxylic, and malonic; 200 μM) as well as HCl (200 μM) did not suppress endothelial cell replication. Oxalate also inhibited endothelial cell migration but had no effect on basal, thrombin-induced, or arachidonate-induced prostacyclin production by endothelial cells. Exposure of endothelial cells to sodium oxalate (200 μM) for as little as 24 hours—a time period sufficient to induce delayed, transient inhibition of replication not detectable until approximately 1 week after exposure—inhibited incorporation of 3H-leucine into protein by 40% (p=0.009). We conclude that sodium oxalate acts as an uremic toxin, inhibiting endothelial cell replication and migration, functions which may be important for constitutive inhibition of atherosclerosis. (Arteriosclerosis 10:198–207, March/April 1990)

Physical and metabolic integrity of the endothelium may be critical in preventing or retarding atherosclerosis,32 and therefore, agents that either injure the endothelium or inhibit endothelial cell functions may foster atherogenesis. Oxalic acid is a dicarboxylic acid, which in humans is the end product of the oxidative metabolism of both ascorbic and glyoxylic acids. It is a chelator with no known function in humans but is an inhibitor of intermediary metabolism in cellular and cell-free systems in vitro.33-36 Oxalate is excreted entirely by the kidney and therefore rises in plasma in proportion to the decline in glomerular filtration.37,38,39 Because dialysis does not normalize oxalate concentrations in the plasma of patients with chronic renal failure,37,38,39 elevated plasma oxalate levels may be one of the atherogenic factors present in chronic uremia. We therefore studied the ability of oxalate to alter endothelial function as an in vitro model of the "response-to-injury hypothesis."32 In this paper, we show that elevated oxalate levels inhibit endothelial cell replication and migration.

Methods

Cell Culture

Human umbilical vein endothelial cells were obtained and cultured as previously described.40 In brief, cells were harvested from umbilical veins with collagenase (0.1%) and maintained in Medium 199 supplemented with 20% pooled human serum to which amphotericin B (2 μg/ml), L-glutamine (1.6 mM), penicillin (40 U/ml), and
streptomycin (40 μg/ml) were added. Cells from single primary cultures at confluence were plated in 2 to 8 replicate subcultures containing identical cell numbers in T-25 culture flasks or 6- or 24-well plates (Corning, Corning, NY). The medium was changed every 3 days, and test materials were added with each change of medium. The conditions applied to subsequent passages were described below. Human lung fibroblasts (Human Genetic Mutant Cell Repository, Rockville, MD), passage 13, were cultured in minimal essential medium (Flow, McLean, VA) with 20% fetal calf serum to which the antibiotics listed above were added.

Quantification of Cell Replication

Cell replication was defined as the increase in the number of cells in a given culture over time. The effects on cell replication of sodium oxalate (0 to 300 μM), other carboxylic and organic acids (citric, succinic, glyoxylic, malonic, and hydrochloric; 0 to 200 μM; Sigma, St. Louis, MO), and other metabolites that accumulate in renal failure (creatinine 0 to 1.1 mM; urea 0 to 25 mM) alone and in combination were determined by comparing cell replication in subcultures derived from a single primary culture.

Cells were counted in situ daily by minor modifications of a morphometric technique previously described. At the start of each experiment, four to six permanent counting sites defined by a template were marked on the undersurface of each flask. The marks allowed absolute positioning of every flask, so that identical fields (same size and same relative position within each flask) could be counted repeatedly. Depending on the experiment, all cells in a 200×, 300×, or 400× field visualized by phase contrast in an inverted microscope (Nikon, Garden City, NY) were manually counted by using a 90° reticle as a counting aid. The technique yields counts that are tightly correlated with counts obtained manually by protocol 3. Since in all experiments, regardless of the protocol used, oxalate caused significant inhibition of cellular replication, the results from all protocols have been combined in the data presented.

Labeling of Endothelial Cells with 51Cr

For short-term experiments in which chromium release was studied, endothelial cells were incubated in medium containing 51Cr (sodium chromate, 5 μCi/ml; New England Nuclear, Boston, MA) for 3 hours and washed twice before use. For long-term experiments, cells were incubated with 51Cr for 24 hours.

Preparation of Cells for Transmission Electron Microscopy

Cells were washed twice with Medium 199 without human serum and were detached from flasks by incubation at 37°C for 15 minutes with 0.1% ethylenediaminetetraacetic acid and 0.1% collagenase (Type I, Worthington, Freehold, NJ) in a buffer containing 10 mM HEPES (pH 7.4 at 37°C), 137 mM NaCl, 5 mM KCl, and 5.5 mM glucose. Cells were subsequently treated as previously described for viewing in an Elmiskop 1 (Siemans, Iselin, NJ).

Radioimmunoassay for 6-keto-PGF₁α

Endothelial cells were grown in medium alone or in medium containing sodium oxalate (0 to 200 μM) for various times. The cells were fed with fresh medium containing appropriate concentrations of oxalate for 24 hours before stimulation. The cells were washed twice with HEPES-buffered saline (10 mM HEPES [pH 7.4 at 37°C], 137 mM NaCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM KCl, and 5.5 mM glucose) and were stimulated with 20 μM sodium arachidonate for 2 minutes or with human thrombin (0.1 U/ml) for 5 minutes, since prostacyclin (PGI₂) production was maximal at these times for these agonists. Supernates were removed, snap-frozen, and subsequently analyzed by specific radioimmunoassay for
the stable metabolite of PGI₂, 6-keto-PGF₁α, as previously described.45

Effect of Oxalate on Endothelial Cell Migration

Confluent monolayers of endothelial cells were wounded with a razor blade according to the method of Schleef and Birdwell in 60 minutes after being treated with mitomycin C (10 μg/ml). The distance of cellular migration into the wound approximately 70 hours after wounding was determined by using phase-contrast microscopy and aligning the original edge of the wound with the "O" line on a 10 x 10 graticule and counting the number of boxes from the line containing cells in each of the 10 rows defined by the reticle.

Effect of Oxalate on Macromolecular Incorporation of ³H-Leucine and ³H-Thymidine

Endothelial cells were exposed to medium alone or to medium containing sodium oxalate (200 μM) for 24 hours. Either ³H-leucine (10 μCi/ml) or ³H-thymidine (7 μCi/ml; both New England Nuclear) was then added to the medium, and the cells were labeled overnight. In some experiments, cells were pretreated with cycloheximide (3 μg/ml). After labeling, the cells were washed three times in cold, HEPES-buffered saline. The cells were disrupted with three cycles of freeze-thawing, the macromolecules were precipitated with 10% trichloroacetic acid, and the pellets and supernates were counted in a liquid scintillation counter (Tri-carb 4500, Packard, Sterling, VA). Since both uptake of label into cells (for ³H-leucine: 7.8±2.5% in control vs. 9.1±3.6% in oxalate-treated cultures [n=9, p>0.5]; for ³H-thymidine: 10.1±5.2% in control vs. 13.8±6.9% in oxalate-treated cultures [n=9, p>0.5]), and cell counts were identical in control and oxalate-treated cultures, the protein and DNA synthesis were expressed as the ratios of counts between the precipitate and supernatant fractions obtained from the cell lysates.

Statistical Analysis

The significance of the effect of various treatments and their interactions on cell replication was determined by analysis of variance (ANOVA) or repeated-measures ANOVA as appropriate, and post-hoc pairwise comparisons were made with the least-significant-difference test. The significance of the effect of sodium oxalate on endothelial cell PGI₂ production was also determined by ANOVA. The significance of the effect of sodium oxalate on endothelial cell migration, a noncontinuous variable, was determined by the Mann-Whitney U test. Analyses were performed by computer programs contained either in the Hewlett-Packard statistics library (09815-15001) or in PC Statistician/PC ANOVA (Human Systems Dynamics, Northridge, CA). Data are presented as mean±SD unless stated otherwise.

Results

Effect of Sodium Oxalate on Cellular Replication

Sodium oxalate significantly inhibited endothelial cell replication in a time-dependent manner. In each experiment, the cells were counted repeatedly in one to four flasks for each condition, and within each flask at four to six sites when T-25 flasks were used and six sites when T-75 flasks were used. As shown in Figure 1A, the replication-retarding effect of oxalate was usually detectable after 1 week of incubation, while the earliest significant difference in any experiment was seen at 3 days. As shown in Figure 1B, the effect of sodium oxalate was both dose- and time-dependent (both p<0.0001); in some experiments, prolonged exposure to oxalate led to the death of the culture. When the mean cell counts from 17 experiments were analyzed, exposure of human endothelial cells to sodium oxalate (mean±SD, 182±38 μM; range, 30 to

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The effect of sodium oxalate on human endothelial cells. A. Human umbilical vein endothelial cells in passage 1 were cultured in medium alone or in medium containing 150 μM of sodium oxalate. Cells were counted in situ at six identical sites in two replicate flasks for each condition at the times indicated. Retardation of replication induced by oxalate was evident by Day 11. By Day 20, oxalate induced a 71% reduction in cell density, from an average of 2133±199 to 618±167 total, cumulative cell counts/300x field (p=0.0001). A representative experiment is shown in which four replicate subcultures of endothelial cells were exposed to the concentrations of oxalate shown. Both time and oxalate concentration affected cell count (both p<0.0001). By 25 days, 30 μM of oxalate had inhibited replication by 21% from a total, cumulative cell count of 9120±1659 to 7248±1879 (p<0.05); 100 μM oxalate by 36% (to 5808±559 cells, p<0.01); and 300 μM by 42% (to 5312±1322 cells, p<0.01).
Figure 2. Reversibility of oxalate-induced retardation of replication. A primary culture of human umbilical vein endothelial cells was divided into eight replicate subcultures and was grown in medium alone or in medium containing 200 \( \mu \)M of sodium oxalate for 0 to 17 days. The cultures were exposed to medium containing oxalate for 0, 1, 3, 5, 10, 12, or 15 days as shown and were then grown in medium alone for the remainder of the experiment. By Day 12, the subculture never exposed to oxalate had a total, cumulative cell count of 312±61 cells/300x field. In contrast, by Day 12, subcultures exposed to oxalate for any and all times shown were inhibited by an average of 62% (to 118.5±13.8 cells/300x field, \( p<0.0001 \)). Cultures exposed to oxalate for 1, 3, or 5 days showed delayed inhibition of replication, which began on Day 8, persisted through Day 12, and then abated so that by Day 15, these subcultures contained cell counts that were virtually identical to the subcultures never exposed to oxalate (274.1±10.6 vs. 276.8±67.1, respectively). However, by Day 15, the cell counts in subcultures exposed to oxalate for 8, 10, 12, or 15 days remained significantly different from the controls (mean=127.7±20.5, \( p<0.0001 \)). This pattern persisted through the end of the experiment, i.e., to Day 17.

Further, when each of the 17 experiments was analyzed individually, oxalate significantly inhibited cellular replication in every experiment.

Figure 2 demonstrates that the replication-retarding effect of oxalate was reversible and that reversibility depended on the length of exposure. Exposure to 200 \( \mu \)M of oxalate for as little as 24 hours and for up to 5 days resulted in a significant, but delayed and transient, inhibition of cellular replication (\( p<0.0001 \)). This inhibition persisted until the 15th day, at which time cell counts in these subcultures, which had been exposed to oxalate for up to 5 days, were virtually identical with the cell count in the control subculture, which was never exposed to oxalate. In contrast, subcultures exposed to oxalate for longer than 5 days had not recovered by the termination of the experiment at Day 17 and demonstrated persistent inhibition of cellular replication. Virtually identical results were obtained in two additional experiments.

To determine the specificity of this phenomenon, several experiments were performed. As shown in Figure 3, 200 \( \mu \)M of oxalate had no effect on the replication of fibroblasts studied for 45 days in culture. Thus, oxalate is not a general cellular toxin. To determine whether inhibition of cell replication is a general property of organic acids, endothelial cells were exposed to several carboxylic acids (Figure 4). While exposure of endothelial cells to 200 \( \mu \)M of oxalate for 18 days resulted in a 38% inhibition of cell density (5664±1105 vs. 9024±817, \( p<0.0001 \)), exposure to all of the other carboxylic acids tested (citric, succinic, glyoxylic, and malonic) did not alter cellular replication. In other experiments, neither HCl nor sodium glycolate (each 200 \( \mu \)M) altered endothelial cell replication (data not shown). Thus, oxalate appears to have a specific inhibitory effect on cell replication not shared by the other acids tested.

Calcium Chelation and Inhibition of Cellular Replication by Sodium Oxalate

Sodium oxalate binds ionized calcium and, while it is unlikely that chelation of 200 \( \mu \)M of calcium in a medium containing 1.8 mM calcium would be of significance in contributing to the inhibition of cellular replication, we studied this directly. Four replicate subcultures from a
single primary culture were grown, respectively, in medium alone, medium containing 200 μM sodium oxalate, medium to which 200 μM calcium chloride had been added, and medium containing 200 μM sodium oxalate to which 200 μM calcium chloride had been added. Cells were counted repeatedly over a 33-day period at six sites in each flask. Control and calcium-treated cultures exhibited virtually identical growth curves with mean, cumulative cell counts on Day 33 of 35,586±4420 and 36,288±3267, respectively (p=0.74) between the total, cumulative cell count in the control culture (59,400±166,085) or in the culture exposed to oxalate (68,750±183,520).

Effect of Sodium Oxalate on 61Cr Release from Endothelial Cells

While the replication curves do not suggest that oxalate has an acutely toxic, lethal effect, we studied this possibility directly by determining whether sodium oxalate enhanced the release of 61Cr from endothelial cells. Sodium oxalate had no effect on 51Cr release from endothelial cells after exposure for 0 to 4 hours or 1 to 10 days (data not shown), suggesting that the inhibition of replication is, in fact, due to slowing of replication rather than to increased cell death.

Morphological Characterization of Endothelial Cells Treated with Sodium Oxalate

Elevated concentrations of oxalate in both hereditary oxalosis and chronic uremia are associated with the formation of oxalate crystals, which may be associated with vasculitis.47–49 We explored the possibility that crystal formation or destruction of intracellular organelles might be responsible for retardation of replication by microscopic observations of cellular morphology. Within a week to 10 days of growth in medium containing sodium oxalate (200 μM), there were no differences noted in morphology of cells viewed under phase-contrast microscopy compared to cells grown in medium alone, and no crystals were observed under polarizing microscopy. As cultures aged, they developed a typical "senescent" appearance with many giant cells, large nuclei, and extensive vacuolization; these changes appeared much earlier in oxalate-treated than in control cultures. No other obvious morphologic differences at the level of light microscopy between cells exposed to medium alone or to medium containing sodium oxalate were noted. The absence of crystal formation and intracellular disruption was confirmed by transmission electron microscopy (data not shown).

Interaction of Sodium Oxalate with Other Metabolites that Accumulate in Renal Failure

Since urea is known to enhance the solubility of oxalate,50 we determined whether uremic concentrations of these two metabolites interacted in altering endothelial cell replication. We also studied the interaction of oxalate and creatinine. As shown in Figure 5A, by Day 26 in culture while both urea (25 mM or 150 mg/dl [equivalent to a blood urea nitrogen level of approximately 70]) and oxalate (150 μM) alone suppressed endothelial cell replication (by 22% and 66%, respectively, both p<0.0001), their combination was additive (90% suppression). This result was obtained in two of three experiments, while in the third, urea alone did not inhibit cell replication, and there was no additive effect of oxalate and urea in inhibiting cell replication. As shown in Figure 6B, creatinine (1.1 mM or 12.4 mg/dl) and oxalate also independently inhibited endothelial cell replication (by 11% and 84% respectively, both p<0.001), and their combination was additive (99% suppression). Similar to the variability described above for the effect of urea, the inhibitory effect of calcium by oxalate is responsible for the oxalate-induced inhibition of cellular replication.
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Figure 4. Specificity of sodium oxalate as an inhibitor of cell replication. A primary culture of endothelial cells was divided into six replicate subcultures and was grown in medium alone or in medium containing 200 μM of the sodium salts of the carboxylic acids listed. At Day 16, the total, cumulative cell counts/300× field in each of these subcultures were: control, 9024±897; oxalate, 5664±1105; citrate, 9824±1049; succinate, 10404±1603; glyoxylate, 8976±1627; and malonate, 9528±891. The inhibition of cellular replication induced by oxalate was significant (p<0.0001), and cell counts in the oxalate subculture differed significantly from each of the other subcultures (all p<0.01), while none of the cell counts in the subcultures exposed to the other acids differed significantly from control or each other.

of creatinine was seen in only one of two experiments. Thus, both urea and creatinine, independently, are weak inhibitors of endothelial cell replication compared to oxalate, and their interactions with oxalate are variable.

Effect of Sodium Oxalate on Endothelial Cell Prostacyclin Production

To determine whether oxalate also altered endothelial function, as represented by eicosanoid synthesis, the effect of sodium oxalate on PGI₂ production by endothelial cells was determined both in the basal state and after stimulation by thrombin and sodium arachidonate. Table 1 demonstrates that exposure of near-confluent endothelial cells to various concentrations of sodium oxalate (0 to 200 μM) for 4 days had no effect on PGI₂ production.

Similarly, when the endothelial cell conditioned media from four different cultures grown with or without oxalate were examined serially (three to six changes of medium spanning 15 to 24 days) and the production of PGI₂ was normalized for cell count, sodium oxalate had no effect on PGI₂ production (data not shown). Thus, the complex cascade of events that generates PGI₂ from arachidonic acid was not altered by prolonged exposure to oxalate.

Effect of Sodium Oxalate on Endothelial Cell Migration After Wounding

Figure 6 shows that sodium oxalate inhibits migration of endothelial cells. After exposure to 200 μM of sodium oxalate for 10 days, migration 70 hours after wounding the monolayer was inhibited by 45% (p<0.0001). Similar to the time requirement for inhibition of endothelial cell replication, inhibition by oxalate of endothelial cell migration was also time-dependent, being present after a 10- but not a 3- or 7-day exposure.

Effect of Sodium Oxalate on Incorporation of ³H-Leucine and ³H-Thymidine

Since there were no gross morphological abnormalities in endothelial cells exposed to sodium oxalate that might explain its replicative inhibitory effect, we sought to determine whether oxalate altered protein or DNA synthetic activity as a mechanism of retardation of replication. Because exposure to oxalate for as little as 24 hours inhibits cellular replication (see Figure 2) and because substantial reductions in synthetic rate would be expected late in the course of an experiment when the rate of cell replication is dramatically reduced, we studied the effect of sodium oxalate (200 μM) on synthetic rates after only a 24-hour exposure. As shown in Table 2, sodium oxalate inhibited leucine incorporation by approximately 40% (p<0.0001) but decreased thymidine incorporation by only 32%, a change that was not statistically significant (p=0.071). Thus, it is possible that oxalate inhibits endothelial cell replication by interfering with protein synthesis.

Discussion

In this study, we have shown that elevated levels of oxalic acid, an end-product of human metabolism that
accumulates in plasma in renal failure, inhibits both replication and migration of human endothelial cells in tissue culture. Denuding injury of the vascular intima results in atherosclerosis in experimental animals and rapid proliferation of smooth muscle in humans. Further, although the normal rate of division of endothelium in vivo is quite slow, it is probably much more rapid both at branch points where atherosclerosis is more likely to develop and after early atherosclerotic lesions have, in fact, developed. Thus, the ability of surrounding endothelium to quickly resurface the injured area may be important in retarding atherogenesis. In this context, it is of interest that hyperglycemia, the metabolic derangement of diabetes, which is a major risk factor for atherosclerosis, has also been shown to inhibit endothelial cell replication and metabolism. Therefore, the inhibition in vitro of endothelial replication and migration by concentrations of sodium oxalate, which are present in uremic patients undergoing chronic dialysis, may be viewed as a potentially atherogenic phenomenon that may contribute to the increased prevalence and acceleration of atherosclerosis in the uremic population.

Normal plasma oxalate concentrations determined by modern techniques, including in vivo isotope dilution studies, range between approximately 1 and 5 μM. In renal failure, oxalate concentration rises linearly in proportion to the creatinine and therefore is dependent on the severity of renal failure. In patients undergoing chronic dialysis, pre-dialysis serum or plasma oxalate levels are approximately 20 to 200 μM and immediate, post-dialysis levels are approximately 10 to 50 μM. Thus, the concentrations of oxalate utilized in our studies, 30 to 300 μM, encompass the concentrations of oxalate present in uremic patients undergoing chronic dialysis and induce a dose-dependent inhibition of endothelial cell replication (Figure 1). Further, oxalate and urea were additive in inhibiting cell replication in some experiments (Figure 5), and in vivo both metabolites were present continuously in abnormally high concentrations in chronic renal failure. Whether this additive effect is due to the solubilizing effect of urea on calcium oxalate is unknown.

The mechanism by which oxalate inhibits cell replication is probably complex; however, it is apparently not related to chelation of calcium. While both citrate and oxalate bind calcium and have approximately equal affinity constants for calcium, oxalate inhibits endothelial cell replication, while citrate does not (Figure 4). Further-
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Table 1. Effect of Sodium Oxalate on Stimulated Prostacyclin Production

<table>
<thead>
<tr>
<th>Agent</th>
<th>Oxalate concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na A</td>
<td>0</td>
</tr>
<tr>
<td>Thrombin</td>
<td>3.2±0.4</td>
</tr>
</tbody>
</table>

Values of PGF2α are given in ng/0.1 ml.

Table 2. Effect of Sodium Oxalate on Incorporation of [3H]-Leucine and [3H]-Thymidine by Endothelial Cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]-leucine</td>
<td>Control</td>
<td>Oxalate</td>
</tr>
<tr>
<td>4.1±3.5</td>
<td>2.5±2.4</td>
<td>39.8</td>
</tr>
<tr>
<td>[3H]-thymidine</td>
<td>10.3±4.5</td>
<td>7.1±4.0</td>
</tr>
</tbody>
</table>

The results are the means±SD of three experiments of three replicates each.

Endothelial cells were exposed to medium alone or to medium containing sodium oxalate (200 µM) for 24 hours. Either [3H]-leucine (10 µCi/ml final concentration) or [3H]-thymidine (7 µCi/ml) was added, and cells were labeled overnight. Protein and DNA synthesis were expressed as the ratio of counts between the trichloroacetic acid-precipitate and the supernatant fractions of the cell lysates.

more, replacing the calcium that would have been bound by a 1:1 molar complex of oxalate and ionized calcium by the addition of calcium chloride did not reverse the inhibition of cellular replication. In contrast, exposure of endothelial cells to oxalate for 24 hours resulted in an immediate and significant decrease in protein synthesis (Table 2), which was followed in several days by an inhibition of cell replication (Figure 2). Thus, inhibition of protein synthesis by oxalate may result in inhibition of cell replication.

Oxalate has been shown to inhibit lactate dehydrogenase, as well as pyruvate kinase and monophosphoglycerate mutase in both cell-free and intact cell systems. Whether inhibition of glycolytic enzymes is responsible for the inhibition of both protein synthesis and endothelial cell replication is under investigation. However, it is intriguing that just as malonate, the 3-carbon analogue of oxalate, did not inhibit cellular replication (Figure 4), Beutler et al. found that malonate also did not inhibit red cell enzymes of glycolysis.

Additional possibilities for the molecular mechanism of action of oxalate, which are merely speculative, include the modulation of one or more cytokines. Replication of endothelial cells in vitro can be modulated by a host of factors. Stimulants include endothelial cell growth factor and basic fibroblast growth factor (which also stimulates migration); inhibitors include transforming growth factor-beta, gamma-interferon, tumor necrosis factor alpha, lymphotixin, and high glucose concentrations. Since endothelial cells synthesize and secrete, or can be induced to synthesize and secrete, some of these factors, it is possible that oxalate could interfere with cellular replication by altering their synthesis. This question is also under investigation.

The use of short-term endothelial cultures as an in vitro model for derangements that may be present in chronic uremia over years has obvious limitations. Beyond the gross difference in the time scale, the endothelial cells in culture experience a "replication stress" not present in vivo, and there are innumerable differences in the chemical and physical environments of the cells in the two circumstances. Nonetheless, the inhibition of cellular replication and migration by oxalate was striking and reproducible across all experiments. Oxalic acid may be an additional and important atherogenic factor in patients with chronic renal failure.

Acknowledgments

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