Differential effects of EGF on repair of cellular functions after dichlorovinyl-L-cysteine-induced injury

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Nowak, Grażyna, Kenneth B. Keasler, Douglas E. McKeller, and Rick G. Schnellmann. Differential effects of EGF on repair of cellular functions after dichlorovinyl-L-cysteine-induced injury. Am. J. Physiol. 276 (Renal Physiol. 45): F228–F236, 1999.—This study examined the repair of renal proximal tubule cellular (RPTC) functions following sublethal injury induced by the nephrotoxicant S-(1,2-dichlorovinyl)-L-cysteine (DCVC). DCVC exposure resulted in 31% cell death and loss 24 h following the treatment. Monolayer confluence recovered through migration/spreading but not proliferation after 6 days. Basal, uncoupled, and ouabain-sensitive oxygen consumption (QO2) decreased 47, 76, and 62%, respectively, 24 h after DCVC exposure. None of these functions recovered over time. Addition of epidermal growth factor (EGF) following DCVC exposure did not prevent decrease in basal, uncoupled, and ouabain-sensitive QO2 values and Na+-K+-ATPase activity but promoted their recovery over 4–6 days. In contrast, no recovery of Na+-dependent glucose uptake occurred in the presence of EGF. These data show that: 1) DCVC exposure decreases mitochondrial function, Na+-K+-ATPase activity, active Na+ transport, and Na+-dependent glucose uptake in sublethally injured RPTC; 2) DCVC-treated RPTC do not proliferate nor regain their physiological functions in this model; and 3) EGF promotes recovery of mitochondrial function and active Na+ transport but not Na+-dependent glucose uptake. These results suggest that cysteine conjugates may cause renal dysfunction, in part, by decreasing RPTC functions and inhibiting their repair.

renal proximal tubular cells; cysteine conjugate; S-(1,2-dichlorovinyl)-L-cysteine; sublethal cell injury; regeneration; cell repair; mitochondrial function; oxygen consumption; sodium-potassium adenosine triphosphatase; active sodium transport; sodium-coupled glucose uptake; ascorbic acid; epidermal growth factor

THE KIDNEY has the potential for complete recovery from acute renal failure (ARF) following toxicant- and ischemia/reperfusion-induced injury (5, 8). ARF is often associated with tubular damage and cell death, particularly of renal proximal tubular cells (RPTC), due to their active transport functions and selective accumulation of xenobiotics. However, renal dysfunction may be due also to sublethal injury of the tubular epithelium that is associated with the loss of RPTC physiological functions without producing necrosis (35). The most common RPTC alterations observed following toxicant exposure or ischemia/reperfusion are 1) loss and/or internalization of the brush-border membrane microvilli, 2) loss of cell polarity, 3) mitochondrial dysfunction and ATP depletion, 4) inhibition of the Na+-K+-ATPase, and 5) alterations in ion homeostasis and transport functions (1, 2, 21, 35, 39).

Pathological changes in RPTC can be repaired over time, and the return of RPTC physiological functions is critical for the restoration of normal renal function (35, 36). Numerous studies suggest that tubular repair is composed of several steps and begins with the exfoliation of dead cells from the basement membrane. This is followed by the migration/spreading of noninjured cells into the denuded area, dedifferentiation, proliferation, migration, and differentiation of RPTC, ultimately resulting in relining of the damaged tubule.

Epidermal growth factor (EGF) is a potent mitogen for RPTC and is synthesized within the kidney (12, 23, 25, 29). In addition to its well-documented mitogenic activity, EGF exerts effects on a number of important cellular responses including oxygen consumption, glycolysis, gluconeogenesis, arachidonic acid metabolism, intracellular calcium levels, glycosaminoglycans and collagen synthesis, ion exchange, and tubular transport (4, 10, 18, 25, 34). Endogenous EGF levels increase in the kidney following ARF, enhance renal tubule cell regeneration and repair, and promote the recovery of renal function in postischemic ARF (12, 24, 31, 32).

Halogenated hydrocarbons represent a large group of compounds that are used as chemical intermediates, solvents, and pesticides and produce toxicity after their enzymatic conversion to reactive intermediates. Haloalkanes and haloalkenes (e.g., trichloroethylene) are biotransformed in a series of steps that ultimately result in the formation of nephrotoxic cysteine S-conjugates [e.g., S-(1,2-dichlorovinyl)-L-cysteine (DCVC)]. In the RPTC, these conjugates are biotransformed by cysteine conjugate β-lyase to thiol-containing reactive metabolites that produce nephrotoxicity by their covalent binding to target molecules within the cell (6). DCVC is a model halocarbon nephrotoxic that is selective for RPTC and produces RPTC necrosis and ARF (7, 33).

Acute exposure of RPTC to DCVC results in thiol depletion, loss of calcium homeostasis, mitochondrial dysfunction and ATP depletion, lipid peroxidation, DNA damage, loss of brush border enzymes, and inhibition of transport functions (16, 17). These changes are associated with the reorganization of RPTC cytoskeleton including the loss and depolymerization of F-actin, loss of actinin, redistribution of talin, and disturbance of...
focal adhesions and are followed by cell detachment and death (38). Long-term exposure of LLC-PK1 cells to low concentrations of DCVC results in cellular dedifferentiation characterized by alterations in cellular morphology, composition of nuclear matrix and intermediate filament proteins, loss of membrane polarity, and impairment of apical glucose uptake and pH-dependent ammonia production (37).

Recently, we have demonstrated that primary cultures of RPTC grown in improved culture conditions that promote oxidative metabolism and transport functions of RPTC (26) undergo complete morphological regeneration of the monolayer following sublethal injury induced by an oxidant [tert-butyl hydroperoxide (TBHP)] and that this process is due to both proliferation and migration/spreading (27, 28). Furthermore, the decrease in oxidative metabolism, ATP content, Na\(^+\)-K\(^+\)-ATPase activity, active Na\(^+\) transport, and Na\(^+\)-coupled glucose uptake observed after RPTC exposure to TBHP is followed by repair and complete recovery of RPTC functions with cellular proliferation and monolayer regeneration preceding the recovery of mitochondrial and transport functions (28). Thus RPTC cultured in these conditions are able to undergo a complete morphological and functional repair following sublethal oxidative injury.

Numerous studies have demonstrated inhibitory effect of DCVC exposure on RPTC physiological functions. However, the ability of RPTC to restore these functions following DCVC-induced injury has not been examined. It is also unknown whether EGF can promote the repair of RPTC functions following DCVC-induced injury. Therefore, the aim of this study was 1) to determine the effect of DCVC on mitochondrial and brush-border and basolateral membrane functions in RPTC; 2) to determine whether RPTC recover these functions following sublethal injury induced by DCVC; and 3) to examine whether EGF treatment following DCVC exposure protects against the loss of mitochondrial and transport functions of RPTC and/or promotes recovery of these functions.

**MATERIALS AND METHODS**

Materials. Female New Zealand White rabbits (1.5–2.0 kg) were purchased from Myrtle’s Rabbitry (Thompson Station, TN). DCVC was a generous gift from Dr. T. W. Petry (Pharmacia Upjohn, Kalamazoo, MI) and was synthesized according to the method of Moore and Green (22). L-Ascorbic acid-2-phosphate magnesium salt and cell culture media were obtained from Wako BioProducts (Richmond, VA) and Sigma Chemical (St. Louis, MO), respectively. EGF (recombinant, human) was supplied by R&D Systems (Minneapolis, MN). Methyl α-D-glucopyranoside (MGP), [glucose-\(^{14}\)C(U)] (sp act 282 mCi/mmol), was purchased from DuPont-New England Nuclear (Boston, MA). The sources of the other reagents have been described previously (25, 26).

Isolation of proximal tubules and culture conditions. Rabbit renal proximal tubules were isolated by iron oxide perfusion method and grown in 35-mm culture dishes in improved conditions as described previously (25, 26). The purity of the renal proximal tubular S1 and S2 segments isolated by this method is ~96%. The culture medium was a 50:50 mixture of DMEM and Ham’s F-12 nutrient mix (without phenol red, pyruvate, and glucose) supplemented with 15 mM NaHCO\(_3\), 15 mM HEPES, and 6 mM lactate (pH 7.4, 295 mosmol/kg). Human transferrin (5 µg/ml), selenium (5 ng/ml), hydrocortisone (50 nM), bovine insulin (10 nM), and L-ascorbic acid-2-phosphate (0.05 mM) were added to the medium immediately before daily media change (2 ml/dish).

Toxicant treatment of RPTC monolayer. RPTC cultures reached confluence within 4–5 days and were treated with DCVC on the sixth day. RPTC were treated with 0.2 mM DCVC (dissolved in water; 10 µl/dish) for 1.5 h to obtain ~30% cell death and loss. Following toxicant exposure, the remaining cellular monolayer was washed with fresh culture medium. In some experiments, RPTC were treated daily with EGF (10 ng/ml) starting with the media change following DCVC exposure. In other experiments, RPTC were cultured in the presence of 5 mM glucose with or without EGF (10 ng/ml) following DCVC treatment. Samples of RPTC were taken at various timepoints after exposure for measurements of cellular functions. Prior to measurement of any functions, RPTC were washed several times with warm culture media to remove nonviable cells.

Oxygen consumption. RPTC monolayers were washed with 37°C culture medium and gently detached from the dishes with a rubber policeman, suspended in 37°C culture medium, and transferred to the oxygen consumption (QO\(_2\)) measurement chamber. QO\(_2\) was measured polarographically in RPTC suspended in the culture medium using Clark-type electrode as described previously (25, 26). For measurement of ouabain-sensitive and uncoupled QO\(_2\), 0.1 mM ouabain and 1 µM FCCP (final concentrations) were used, respectively.

Na\(^+\)-coupled glucose uptake. Na\(^+\)-coupled glucose uptake was assessed using the net metabolizable glucose analog MGP as described previously (26). MGP uptake was measured in glucose-free medium used for RPTC culture and corrected for Na\(^+\)-independent (phlorizin-insensitive) and time 0 uptakes.

Enzyme assays. Na\(^+\)-K\(^+\)-ATPase activity was determined in cellular lysates by measuring the difference between total ATPase activity and ouabain-insensitive ATPase activity as described previously (26). γ-Glutamyl transpeptidase (GGT) activity (brush-border membrane marker) was determined in cell lysates according to Meister et al. (20). Protein was measured by the method of Lowry et al. (19). DNA content was determined by the method of Labarca and Paigen (15) as described previously (25).

Statistical analysis. Data are presented as means ± SE and were analyzed for significance using two-way ANOVA or Student’s t-test for paired data where appropriate. Multiple means were compared using Student-Newman-Keuls test. Statements of significance were based on P < 0.05. Renal proximal tubules isolated from an individual rabbit represented a separate experiment (n = 1) consisting of data obtained from three plates.

**RESULTS**

Monolayer regeneration. Exposure of confluent RPTC monolayers to 0.2 mM DCVC for 1.5 h resulted in 17% cell loss, as indicated by DNA content, at 4 h and 31% at 24 h following the treatment (Fig. 1). Monolayer DNA content in DCVC-injured RPTC did not return to control values during the recovery period. The protein-to-DNA ratio decreased at 24 h and returned to control values by day 4 following DCVC treatment (Table 1). Visual inspection demonstrated that monolayer confluence recovered by day 6 as reported previously (14). These results show that monolayer recovery was due to
migration/spreading of sublethally injured RPTC and not proliferation or hypertrophy.

Mitochondrial function. Basal and uncoupled QO₂ were used as markers of RPTC mitochondrial function. Specifically, uncoupled QO₂ served as a marker of electron transport chain integrity. DCVC exposure decreased basal QO₂ in sublethally injured RPTC by 33% and 47% at 4 and 24 h, respectively (Fig. 2A). Uncoupled QO₂ was decreased by 64% and 76% at 4 and 24 h, respectively, after DCVC exposure (Fig. 2B). No significant changes in basal and uncoupled QO₂ occurred in sublethally injured RPTC during the 6-day recovery period following DCVC exposure. Ouabain-insensitive QO₂ decreased by 37% at 24 h (13.3 ± 1.3 vs. 8.7 ± 1.8 nmol O₂·min⁻¹·mg protein⁻¹ in control and DCVC-treated RPTC, respectively) and remained decreased by 20% until day 6 following the exposure. These data show that DCVC treatment decreases mitochondrial function in RPTC and that mitochondrial function in sublethally injured RPTC does not recover over time.

Basolateral membrane function. Active Na⁺ transport was used as a marker of basolateral membrane function. The assessment of active Na⁺ transport in RPTC was done by measurements of ouabain-sensitive QO₂ and Na⁺-K⁺-ATPase activity. DCVC exposure in RPTC resulted in 50% and 62% decreases in ouabain-sensitive QO₂ at 4 and 24 h, respectively (Fig. 3A). Na⁺-K⁺-ATPase activity was inhibited by 62% and 80% at 4 and 24 h, respectively (Fig. 3B). Neither ouabain-sensitive QO₂ nor Na⁺-K⁺-ATPase activity recovered over time (Fig. 3). These data show that DCVC decreases active Na⁺ transport in RPTC and that the repair of this function does not occur in sublethally DCVC-injured RPTC.

Brush-border membrane function. Na⁺-dependent glucose uptake and GGT activity were used as markers of brush-border membrane function in RPTC. Na⁺-dependent glucose uptake was reduced by 25% and 68% at 4 and 24 h, respectively, following DCVC exposure (Fig. 4). No recovery of this function occurred over the 6-day period after DCVC treatment. In contrast, DCVC had no effect on GGT activity (452 ± 45 vs. 437 ± 46 mU/mg protein in DCVC-treated and control RPTC, respectively, at 4 h following the exposure, and 408 ± 33 vs. 457 ± 31 mU/mg protein in DCVC-treated and control RPTC, respectively, at 24 h following the exposure). These data show that DCVC-induced injury is associated with a marked decrease in Na⁺-dependent glucose uptake and lack of recovery of this function. The results also show that not all brush-border membrane proteins are altered by DCVC.

Another aim of this study was to investigate whether EGF can promote recovery of RPTC functions following DCVC-induced injury. Table 1. Protein-to-DNA ratios in renal proximal tubular cells following DCVC (0.2 mM) exposure

<table>
<thead>
<tr>
<th>Time After DCVC Removal</th>
<th>Control</th>
<th>DCVC</th>
</tr>
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<tbody>
<tr>
<td>4 h</td>
<td>64.1 ± 3.5*</td>
<td>62.7 ± 4.4*</td>
</tr>
<tr>
<td>1 day</td>
<td>67.0 ± 7.9*</td>
<td>44.5 ± 2.5†</td>
</tr>
<tr>
<td>4 days</td>
<td>77.1 ± 7.0*</td>
<td>68.8 ± 7.9*</td>
</tr>
<tr>
<td>6 days</td>
<td>72.3 ± 9.4*</td>
<td>66.8 ± 9.2*</td>
</tr>
</tbody>
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Values are means ± SE (n = 6–9). DCVC, S-(1,2-dichlorovinyl)-L-cysteine. Values with different symbols (*, †) are significantly different from each other.
Effect of EGF on recovery of mitochondrial function. The addition of EGF to RPTC monolayers during the first 24 h following DCVC exposure had no effect on DCVC-induced decreases in basal and uncoupled QO₂ (Fig. 5). However, in the presence of EGF, basal and uncoupled QO₂ in DCVC-injured RPTC returned to control values on day 6 (Fig. 5). EGF had no effect on the decrease in ouabain-insensitive QO₂ in DCVC-treated RPTC at 24 h (37% vs. 36% in DCVC-treated RPTC in the absence and presence of EGF, respectively). However, in the presence of EGF, ouabain-insensitive QO₂ in DCVC-injured RPTC returned to control values on day 4 (14.8 ± 0.9 vs. 16.9 ± 1.6 nmol O₂ in the absence and presence of EGF, respectively). These data show that EGF does not protect against DCVC-induced decreases in RPTC respiration but promotes the repair of mitochondrial function in RPTC following DCVC injury.

Effect of EGF on recovery of basolateral membrane function. Figure 6A shows that addition of EGF following DCVC exposure had no effect on the DCVC-induced decrease in ouabain-sensitive QO₂. However, ouabain-sensitive QO₂ in DCVC-injured RPTC cultured in the presence of EGF returned to control levels on day 6 (Fig. 6A). Addition of EGF to DCVC-injured RPTC diminished the decrease in Na⁺-K⁺-ATPase activity on day 4 (Fig. 6B).
that EGF causes further decrease in Na\(^{+}\)-dependent glucose uptake in DCVC-injured RPTC.

Effect of EGF on RPTC proliferation following DCVC injury. Figure 8A shows that administration of EGF following DCVC exposure did not induce RPTC proliferation. This suggested that the lack of RPTC proliferation following DCVC-induced injury may be due to the inability of RPTC to respond to the mitogen or to synthesize DNA from substrates present in the culture medium. Supplementation of the culture medium with glucose (5 mM), a common source of precursors for DNA synthesis in cultured cells (30), following DCVC exposure did not change DNA content in injured monolayers (Fig. 8B). However, EGF treatment in the presence of glucose resulted in RPTC proliferation and complete recovery of monolayer DNA content on day 4 after DCVC exposure (Fig. 8B). These results show that: 1) DCVC-injured RPTC lack a mitogenic signal necessary to induce proliferation following injury, and 2) DCVC-treated RPTC are capable of a mitogenic response and of synthesizing new DNA with glucose supplementation.

DISCUSSION

Renal failure caused by toxicant exposure is often due to RPTC injury, death, and detachment. However, in many cases, toxicant-induced renal failure is associated with RPTC dysfunction without apparent necrosis and cell loss (35). In these conditions, sublethally injured RPTC have decreased physiological functions and the recovery of normal renal function occurs if RPTC repair their functions. There is a possibility, however, that some toxicants may contribute to renal dysfunction not only by inducing RPTC injury but also by inhibiting the repair process. Our previous study...
intracellular proteins in injured RPTC. The results suggested that the recovery of the monolayer confluence following DCVC exposure was due to migration and spreading and remain in contrast to our previous observation that RPTC proliferation occurs following an oxidant (TBHP) exposure that produces a similar amount of RPTC death and loss (28).

The absence of RPTC proliferation following DCVC-induced injury could be due to numerous reasons including the lack of a mitogenic signal, the inability to respond to this signal, damage to the DNA synthesis apparatus and/or inability to generate sufficient DNA precursors from substrates present in the culture medium. To test the hypothesis that the lack of proliferation is due to the absence of a mitogenic signal, we supplemented the culture medium with EGF, a potent mitogen for RPTC in vivo and in vitro (12, 23, 25). EGF did not induce RPTC proliferation during 6 days after DCVC exposure. The absence of proliferation could be due also to the lack of appropriate DNA precursors. To test this hypothesis, we supplemented the culture medium with glucose, a common substrate for DNA precursors in cultured cells (30), and demonstrated that glucose alone did not promote proliferation in DCVC-injured RPTC. However, EGF in conjunction with glucose induced DNA synthesis and proliferation in DCVC-injured monolayers, which suggests that the inability of RPTC to proliferate after DCVC exposure is due to the lack of a mitogenic signal and the appropriate source of DNA precursors. These results also suggest that in this model RPTC maintain their ability to respond to mitogenic signals following DCVC injury and that EGF-stimulated DNA synthesis requires an alternate or additional source of DNA precursors. In contrast, TBHP-induced injury in RPTC is followed by proliferation that does not require the presence of EGF and additional sources of DNA precursors (28).

Mitochondrial function, active Na\(^+\)-K\(^+\)-ATPase activity, and Na\(^+\)-dependent glucose uptake are major targets of DCVC in RPTC. A decrease in these functions was observed immediately after DCVC removal (time 0) from the monolayers, when RPTC did not exhibit any evidence of cell death. Further decreases of mitochondrial function, active Na\(^+\)-transport and Na\(^+\)-K\(^+\)-ATPase activity, and Na\(^+\)-dependent glucose uptake were observed 24 h later when most lethally injured RPTC were dead and lost. A decrease in mitochondrial function in DCVC-injured RPTC is consistent with previous reports that demonstrated a decrease in state 3 respiration, dissipation of the mitochondrial membrane potential, and mitochondrial damage in RPTC subjected to DCVC exposure (9, 38). This study shows that the mitochondrial electron transport chain is a target of DCVC in sublethally injured RPTC and that both electron transport chain integrity and mitochondrial respiration are not repaired over the 6-day recovery period in this model. The lack of repair of mitochondrial function following DCVC-induced injury remains in contrast to the complete recovery of this function after oxidant-induced injury in RPTC (28).
These results also demonstrate that DCVC decreases active Na⁺ transport, the basolateral membrane function that is critical for Na⁺ reabsorption by renal proximal tubules in vivo. This decrease is due, in part, to the inhibition of Na⁺-K⁺-ATPase activity in sublethally injured RPTC. To our knowledge, this is the first report showing the inhibitory effect of DCVC exposure on active Na⁺ transport and Na⁺-K⁺-ATPase activity in renal cells. The mechanism of this inhibition remains to be determined. The decrease in the Na⁺-K⁺-ATPase activity may be due to DCVC binding to Na⁺-K⁺-ATPase protein, as DCVC binds covalently to RPTC proteins (3, 9). It is also likely that DCVC exposure results in the loss of Na⁺-K⁺-ATPase protein from sublethally injured RPTC. It has been shown that DCVC causes depolymerization of F-actin and disorganization of RPTC cytoskeleton (38). As Na⁺-K⁺-ATPase is associated with the cytoskeleton through F-actin, depolymerization of actin may cause a partial loss of Na⁺-K⁺-ATPase protein from injured cells. Independently, the loss of mitochondrial function and the resulting ATP depletion would decrease Na⁺-K⁺-ATPase activity and active Na⁺ transport. The lack of repair of active Na⁺ transport and Na⁺-K⁺-ATPase activity in RPTC following DCVC-induced injury remains in contrast to complete recovery of these functions in RPTC after oxidant injury (28).

DCVC-induced injury in RPTC is associated also with a decrease in Na⁺-dependent glucose uptake, the brush-border membrane function that is critical in vivo for reabsorption of glucose from renal filtrate. Our results obtained using cultured RPTC are in agreement with a previous report that demonstrated impaired reabsorption of glucose by the isolated perfused rat kidney following administration of DCVC in vivo (13). The decrease in Na⁺-dependent glucose uptake in DCVC-injured RPTC may be due to the loss of mitochondrial function, decreased Na⁺ gradient, reduced number of Na⁺-dependent glucose transporters on brush-border membrane, or/and direct impairment of the function or binding properties of Na⁺-dependent glucose transporters by DCVC. Regardless of the mechanism, DCVC-induced decreases in Na⁺-dependent glucose uptake by RPTC persisted, and no repair of this function occurred during the 6-day recovery period following the exposure, whereas Na⁺-dependent glucose uptake completely recovered following TBHP-induced injury (28).

Not all brush-border membrane proteins are targets of DCVC. GGT activity was not altered by DCVC exposure or during the recovery period. Ilinskaja and Vamvakas (13) showed increased activity of brush border enzymes in urine after DCVC exposure in vivo in rats, which suggested that DCVC induced brush-border membrane damage or RPTC loss and lysis into urine.

The effect of growth factors on cellular repair following injury is not limited to stimulation of cellular proliferation. Growth factors may limit cell injury by decreasing the effect of agents that induce the damage and/or promote cell repair by supporting the reestablishment of cell-extracellular matrix interactions and/or cell-cell integrity (11). It has been demonstrated that administration of EGF in vivo accelerates both structural and functional recovery of the kidney following acute renal injury (12, 24, 32). Although the stimulation of proliferation in injured RPTC by EGF is well documented (12, 32), it is not clear whether EGF promotes recovery of other RPTC functions following toxicant-induced injury. Our data demonstrate that addition of EGF after DCVC exposure induced repair of the electron transport chain and resulted in the return of basal QO₂ and ouabain-insensitive QO₂, whereas no repair of these functions occurred in DCVC-injured RPTC cultured without EGF. These results show that EGF does not protect against DCVC-induced damage to the mitochondria but induces the repair of mitochondrial function in RPTC following the injury.

This study also demonstrates that EGF does not affect the decrease in active Na⁺ transport in RPTC following DCVC-induced injury but stimulates return of this function. Ouabain-sensitive QO₂ returned to control levels in DCVC-injured RPTC cultured for 6 days in the presence of EGF but not in cells cultured without this growth factor. The data suggest that the stimulation of repair of ouabain-sensitive QO₂ was due, in part, to the protective effect of EGF on Na⁺-K⁺-ATPase activity during first 24 h following DCVC-induced injury. Subsequently, the return of the Na⁺-K⁺-ATPase activity in RPTC cultured in the presence of EGF was observed on day 4 following DCVC exposure in conjunction with the return of mitochondrial function, and was followed by complete recovery of ouabain-sensitive QO₂ on day 6.

Our results also show that EGF did not stimulate the return of Na⁺-dependent glucose uptake and suggest that EGF does not mediate the repair of this function in sublethally injured RPTC following DCVC exposure. These data indicate that EGF does not promote the repair of all physiological functions in sublethally injured RPTC.

In conclusion, our results show that: 1) DCVC decreases proliferation, mitochondrial function, active Na⁺ transport, Na⁺-K⁺-ATPase activity, and Na⁺-dependent glucose uptake in sublethally injured RPTC; 2) none of these functions return over time, which suggests that DCVC inhibits the repair of RPTC functions in this model; 3) EGF stimulates proliferation of sublethally injured RPTC following DCVC exposure but only in the presence of glucose as the source of DNA precursors; 4) EGF promotes the repair of mitochondrial function and active Na⁺ transport in DCVC-injured RPTC but not Na⁺-dependent glucose uptake; and 5) stimulation of recovery of active Na⁺ transport following DCVC exposure is due, in part, to the protective effect of EGF on Na⁺-K⁺-ATPase activity and stimulation of mitochondrial function in sublethally injured RPTC. Our results suggest that nephrotoxic cysteine conjugates may cause renal dysfunction not only by decreasing RPTC functions but also by inhibiting their repair and that the beneficial effect of EGF in...
sublethally injured RPTC is not limited to the stimulation of proliferation.

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