THE NEPHROTOXIN DICHLOROVINYLCYSTEINE INDUCES EXPRESSION OF THE PROTOONCOGENES *C-FOS* AND *C-MYC* IN LLC-PK1 CELLS— A COMPARATIVE INVESTIGATION WITH GROWTH FACTORS AND 12-*O*-TETRADECANOYLPHORBOLACETATE

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Previous studies in kidney cells showed that S-*(1,2-dichlorovinyl)-L-cysteine (DCVC) induces both direct DNA damage and DNA double-strand breaks by activation of* Ca^{2+}*-dependent endonucleases. The objective of this study was to investigate the effects of DCVC on the expression of the protooncogenes* c-fos *and* c-myc *in cultured kidney cells (LLC-PK1). Supplementation of the incubation medium with 10% FCS after 24 hr incubation in 0.2% FCS resulted in a clear, but comparatively weak induction of the expression of* c-fos *and* c-myc *in LLC-PK1 cells. Addition of 500 μm DCVC to the high serum incubation medium induced a further three-fold increase of the transcript levels. A similar increase in the absolute amount of* c-fos *mRNA was induced by a mixture of growth factors (epidermal growth factor/insulin/ transferrin) and of* c-myc *mRNA with 12-*O*-tetradecanoylphorbolacetate. However, the kinetics of gene expression were different. In the presence of DCVC the expression of* c-fos *and* c-myc *increased continuously in a time-dependent manner during the entire incubation period. In contrast, with growth factors and 12-*O*-tetradecanoyl-phorbolacetate the maximum transcript levels were detected after 0.5 hr (*c-fos*) and 1 hr (*c-myc*), respectively; thereafter, a slight decrease was observed up to the end of the incubation time.*

INTRODUCTION

S-(1,2-dichlorovinyl)-L-cysteine (DCVC) is the key metabolite responsible for the acute and chronic nephrotoxicity and nephrocarcinogenicity of trichloroethene and dichloroacetylene (Reichert et al., 1984; National Toxicology Program, 1987). Trichloroethene and

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2. Key Words: *c-fos*, *c-myc*, growth factors, LLC-PK1 cells, 12-*O*-tetradecanoyl-phorbolacetate, *S*-(1,2-dichlorovinyl)-L-cysteine.

3. Abbreviations: dCTP, deoxycytidinetriphosphate; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DMEM, Dulbecco's modified Eagle medium; EGF, epidermal growth factor; EIT, epidermal growth factor/insulin/transferrin; FCS, fetal calf serum; LDH, lactate dehydrogenase; PBS, phosphate buffered saline; SDS, sodiumdodecylsulfate; TPA, 12-*O*-tetradecanoylphorbolacetate.

Cell Biology and Toxicology, Vol. 9, No. 1, pp. 1–13

ISSN: 0742-2091

dichloroacetylene are biotransformed to DCVC, which is nephrotoxic in vivo and cytotoxic to renal cells (Terracini and Parker, 1965; Lash and Anders, 1986; Chen et al., 1990; Darnerud et al., 1991). DCVC is accumulated in the proximal tubules of the kidney and cleaves to reactive, sulfur-containing intermediates by cysteine conjugate β-lyase, which is present with high activities in the mitochondria and cytosol of the proximal tubule cells (Lash et al., 1986).

Several lines of evidence indicate that the reactive intermediates produced upon cleavage of DCVC by β-lyase react directly with DNA: 1) DCVC is a potent mutagen in *Salmonella typhimurium* (Green and Odum, 1985; Dekant et al., 1986); 2) DCVC induces DNA repair in kidney cells in culture (LLC-PK_1) and induces DNA single-strand breaks in the rat kidney in vivo (Jaffe et al., 1985; Vamvakas et al., 1989); 3) results of a recent study indicate that DCVC acylates the exocyclic nitrogen of adenine, guanine, and cytosine (M. Müller, personal communication). In addition to these immediate genotoxic effects, exposure of LLC-PK_1 cells to DCVC induces impairment of the ability of the mitochondria to sequester Ca^{2+}, followed by increase of the cytosolic Ca^{2+} concentrations (Vamvakas et al., 1990). This disturbance of the intracellular Ca^{2+} homeostasis precedes severe perturbations of the mitochondrial membrane potential and cell death. Elevated cellular Ca^{2+} concentrations activate Ca^{2+}-dependent endonucleases; this leads to increased formation of DNA double-strand breaks and increased poly(ADP-ribosyl)ation of nuclear proteins (Vamvakas et al., 1992a).

Both the direct interactions of DCVC derived electrophiles with the DNA and the induction of DNA double-strand breaks may flow into modulation of gene expression (Elia et al., 1991). In addition, an increase in the amount of nuclear poly(ADP-ribosyl)conjugates may also alter gene expression by changing structure and function of nuclear proteins (Boulikas, 1991). In spite of the evidence, the effects of DCVC on the expression of genes related to cell growth, differentiation, and malignant transformation have not yet been investigated.

Induction of the expression of the protooncogenes *c-fos* and *c-myc* plays a central role in cell proliferation and probably also in malignant transformation (Littlewood and Evan 1990; Angel and Karin, 1991). Induction of cell proliferation may be the result of a mitogenic stimulus or of a cytotoxic xenobiotic, which induces cell death and regenerative cell replication in the remaining population. In both cases a strictly controlled, time-limited increased expression of *c-fos* and *c-myc* is observed (Norman et al., 1988; Sawczuk et al., 1988; Cowley et al., 1989). In transformed cells, the mechanisms involved in the expression of these protooncogenes are often perturbed, resulting in permanently enhanced transcription which is largely independent of external stimuli (Campisi et al., 1984). In cooperation with other protooncogenes (i.e., members of the ras family), enhanced expression of *c-fos* and *c-myc* may induce malignant transformation (Littlewood and Evan, 1990; Angel and Karin, 1991). Malignant transformation can also be induced by the oncogenic versions v-*fos* and v-*myc*, which are found in the RNA of tumor viruses (Vennstrom et al., 1982; Suarez, 1989).

This study investigates the effects of DCVC on the expression of *c-fos* and *c-myc* in LLC-PK_1 cells. This cell line exhibits many of the enzymatic and transport properties of the proximal tubule cells, which are the target of the nephrocarcinogenic parent compounds trichloroethene and dichloroacetylene (Hull et al., 1976; Gstraunthaler et al., 1985). For comparison, the *c-myc* and *c-fos* levels were monitored in LLC-PK_1 cells exposed to a mixture of growth factors (epidermal growth factor/insulin/transferrin), which induces expression of *c-myc* in several experimental systems (Müller et al., 1984; Dean et al., 1986; Ran et al., 1987). The tumor promotor 12-*O*-tetradecanoylphorbolacetate was used as a positive control in the induction of *c-fos* expression (Angel et al., 1987).

MATERIALS AND METHODS

Chemicals

S-(1,2-dichlorovinyl)-L-cysteine was obtained by synthesis (McKinney et al., 1959). The product was 99% pure, as determined by analytical HPLC. Cell culture reagents were purchased from Gibco BRL (Eggenstein-Leopoldshafen, FRG).

Tissue Culture of LLC-PK_1 Cells

LLC-PK_1 cells (American Type Culture Collection, passage 196-295) were grown in 150-cm^2 tissue-culture flasks in Dulbecco's modified Eagle medium (DMEM), supplemented with 20 mM HEPES, 10% fetal calf serum (FCS), 100 U penicillin/ml, 100 µg streptomycin/ml, 1.7 g $NaHCO_3$/l, and 3 g glucose/l. Unless otherwise indicated the cells were treated as follows: when the monolayers reached approximately 30-40% confluency the DMEM/10% FCS was replaced with DMEM/0.2% FCS. 24 hr later the cells were exposed to DCVC (200 µM or 500 µM) or to a mixture of epidermal growth factor (20 ng/ml), insulin (20 mg/ml) and transferrin (4 mg/ml) or to 300 nM 12-*O*-tetradecanoylphorbolacetate (TPA).

For the determination of cell proliferation and the activity of lactate dehydrogenase (LDH) in the incubation medium, LLC-PK_1 cells ($5x10^4$) were plated in triplicate in 35 mm ø wells (6 wells per plate). The cells were treated in exponential growth as described above and the cell number was determined with a Coulter counter. LDH release into the incubation medium was determined photometrically at 340 nm by adding 50 ml cell free medium to 1 ml phosphate buffered saline containing 1.3 mM pyruvate and 0.2 mM NaDH. The LDH activity was expressed as a percentage, whereby enzyme activities measured in Triton X-100 (0.5%) lysed cells served as the 100% positive control values.

Isolation of Total RNA

At each time-point, the cells were harvested by trypsinization, counted in the Coulter Counter, and then washed twice with Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS). The isolation of total RNA was performed by a modification of the LiCl/urea method of Auffray and Rougeon (1980). The washed cell pellets (3-4 x 10^6 cells) were resuspended in 1.2 ml LiCl/urea buffer (4 M LiCl; 8 M urea), homogenized by hand in a glass homogenizer, and

stored overnight at 4°C. On the following day, the cell lysate was centrifuged at 17,000 x g (30 min, 4°C), the pellet washed once in LiCl/urea buffer and then resuspended in 6 ml of 10 mM Tris/HCl buffer (pH 7.4). After incubation with 30 µl Proteinase K (stock solution 10 mg/ml) for 2 hr at 37°C, the probe was extracted twice with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1, v:v:v). The organic phase was re-extracted once with chloroform/isoamylalcohol (24:1, v:v) and the RNA was precipitated overnight at -20°C with 1/10 volume of 3 M NaOAc and 2 volumes ice-cold ethanol (99.8 %). On the following day the RNA was pelleted, washed once with 70% ethanol, and dried at 37°C for 1-2 hr. The RNA concentration and purity was determined photometrically at 260 nm/280 nm.

Analysis of c-myc, c-fos *and β-actin mRNA Levels*

Samples (10 µg) of total RNA were separated on a 1% agarose/2.2 M formaldehyde gel and the denatured RNA transferred to a nylon membrane (Amersham, Braunschweig). Before the transfer, ethidium bromide stains were performed to check the quantity and quality of the RNA in each lane. The RNA was cross-linked to the membrane by incubation at 80°C for 2 hr. Before the hybridization, the *c-fos*, *c-myc*, and β-actin probes were radiolabeled according to the method of Feinberg and Vogelstein (1983; 1984) with [a-^{32}P]dCTP (Onchor, Gaithersburg, USA) using a random primer DNA labeling system (BRL, Gaithersburg, USA). The specific activity was approximately 5 x 10^8 dpm/µg DNA. To avoid unspecific binding, the nylon membrane was preincubated for 2 hr with the hybridization solution (50% formamide, 5 x SSC, 5 x Denhardt's, 20 mM Na_2HPO_4, 100 mg/ml salmon sperm DNA, 0.5% SDS, and 10% dextrane sulfate). The hybridization with the radiolabelled *v-myc* or *v-fos* probes was then performed at 42°C overnight. The blots were then washed twice with 2 x SSC and once with 0.1 x SSC/0.1% SDS (1 hr, 55°C), exposed to photographic film (Kodak, Rochester, NY) for 10 days at -70°C, and developed. After revealing the *c-fos* or the *c-myc* signals, the nylon membrane was hybridized with the radiolabeled β-actin probe as described above. This sequential hybridization procedure was performed to avoid overlapping of the signals, since the *c-fos* and the *c-myc* transcripts differ only by 0.2 kb and 0.3 kb, respectively, from the β-actin transcript.

The intensity of the autoradiographic bands was quantified in a scanner with a computer program (Scanner: One Scanner; Computer: Macintosh IIsi; Apple Computer GmbH, München/Ismaning).

RESULTS

Exponentially growing LLC-PK_1 cells were synchronized at the G_0 phase of the cell cycle by incubation for 24 hr in DMEM containing 0.2% FCS, instead of the routinely used 10% FCS. This reduction in FCS concentration did not cause toxic effects according to the cell morphology and the release of lactate dehydrogenase (LDH) in the incubation medium, which was 4.5 ± 1.2% and 4.9 ± 1.8% for 10% FCS and 0.2% FCS, respectively. In the complete

absence of serum, a slight toxicity was observed after 24 hr, as indicated by abnormal cell morphology and the increase in LDH activity to 11.2 ± 2.5%.

After 24 hr incubation in DMEM/0.2% FCS, total RNA was isolated and assayed for *c-myc* and *c-fos* expression by northern blot analysis. Neither of the transcripts was detectable in the serum-deprived cells. In contrast, monolayers exposed to 10% FCS after the 24 hr starvation period revealed weak, but clearly detectable *c-myc* and *c-fos* transcripts. In presence of serum, the transcript levels were highest at 0.5 hr and 1 hr for *c-fos* and *c-myc*, respectively (Figures 1 and 2), and decreased slightly at the subsequent investigated sampling times up to 5 hr after FCS supplementation (data not shown). Addition of 500 µM DCVC to DMEM/10% FCS induced a marked time-dependent increase in the levels of *c-myc* mRNA between 0.5 hr and 5 hr (Figure 1). Densitometric quantification of the intensity of the autoradiogram bands showed a nearly two-fold increase in the *c-myc* mRNA levels 0.5 hr after initiation of DCVC treatment, compared with the levels obtained from the cells that were only stimulated with 10% FCS (Figure 3). The levels of the *c-myc* transcript increased in a time-dependent manner and reached a maximum after 4 hr exposure to DCVC. Treatment of LLC-PK$_1$ cells with 500 mM DCVC for up to 5 hr was not toxic to the cells according to the LDH concentrations in the incubation medium, which did not differ from the control values. In addition, determination of the cell numbers from control and treated monolayers did not reveal any influence of DCVC on cell proliferation during this treatment time (data not shown).

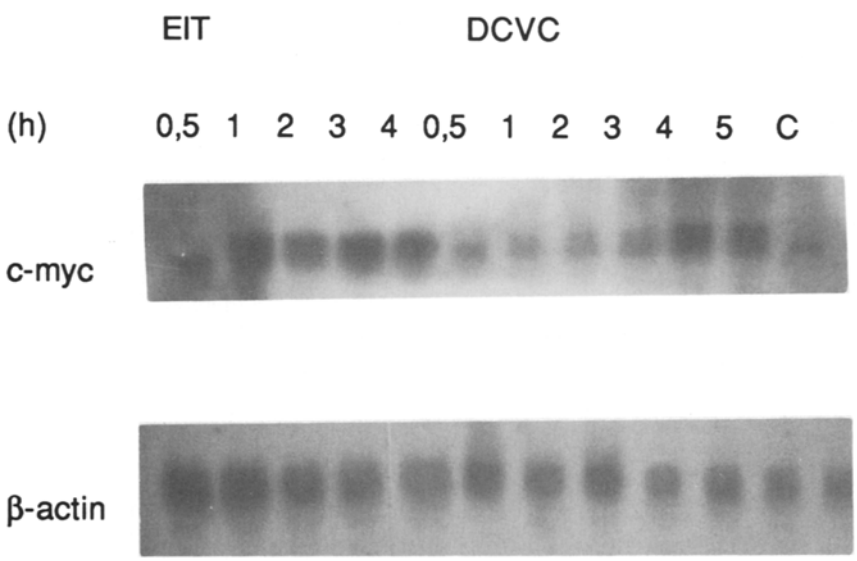

FIGURE 1. Northern blot analysis of *c-myc* and β-actin expression in LLC-PK$_1$ cells. After incubation for 24 hr in DMEM/0.2% FCS, the cells were supplemented with 10% FCS in the presence of 20 ng/ml epidermal growth factor/20 mg/ml insulin/4 mg/ml transferrin (lanes A: 0.5 hr, B: 1 hr, C: 2 hr, D: 3 hr, E: 4 hr) or 500 mM DCVC (lanes F: 0.5 hr, G: 1 hr, H: 2 hr, I: 3 hr, K: 4 hr, L: 5 hr); lane M: control, 1 hr incubation in DMEM/10% FCS after 24 hr serum deprivation (DMEM/0.2% FCS).

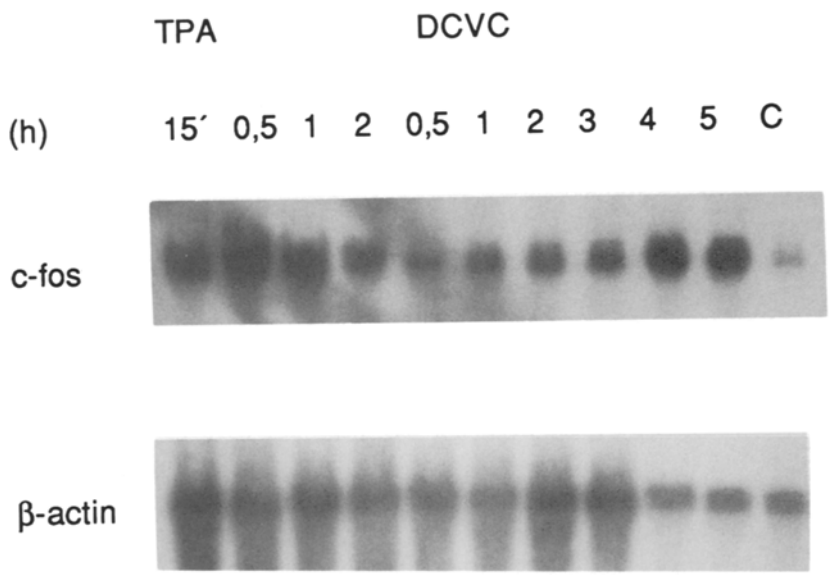

FIGURE 2. Northern blot analysis of *c-fos* and β-actin expression in LLC-PK$_1$ cells. After incubation for 24 hr in DMEM/0.2% FCS, the cells were supplemented with 10% FCS in presence of 300 nM TPA (lane A: 15 min, B: 30 min, C: 1 hr, D: 2 hr) or 500 mM DCVC (E: 0.5 hr, F: 1 hr, G: 2 hr, H: 3 hr, I: 4 hr, K: 5 hr); lane L: control, 0.5 hr incubation in DMEM 10% FCS after 24 hr serum deprivation (DMEM/0.2% FCS).

Exposure of the cells to a mixture of EGF, insulin and transferrin (EIT) caused a similar increase in the absolute amount of the *c-myc* transcript as DCVC (Figures 1 and 2). However, with EIT a different time-pattern was observed. The maximum levels were expressed 1 hr after addition of EIT, and a slight decrease was observed up to the 4 hr time-point.

DCVC at a concentration of 500 μM also induced an increased expression of the *c-fos* mRNA. The *c-fos* transcript was detectable 0.5 hr after addition of DCVC, thus preceding the expression of *c-myc* which started 1 hr after initiation of treatment. The increase was time-dependent and reached a maximum at the 4 hr sampling time (Figure 2). Densitometric analysis of the bands intensity showed that the levels of the *c-fos* mRNA in the DCVC exposed cells were approximately three-fold higher, compared to the monolayers that were stimulated only with serum. The tumor promotor 12-*O*-tetradecanoylphorbolacetate (TPA) has been shown to increase the *c-fos* expression in several experimental models (Angel et al., 1987). In LLC-PK$_1$ cells, 300 nM TPA induced an increase of the *c-fos* transcript, which was highest at the 0.5 hr sampling time and decreased slightly after 1 hr and 2 hr exposure to TPA (Figure 4). Although the time pattern of the TPA-induced *c-fos* expression was different than the one obtained with DCVC, the maximum absolute levels of the transcript were practically the same for both compounds.

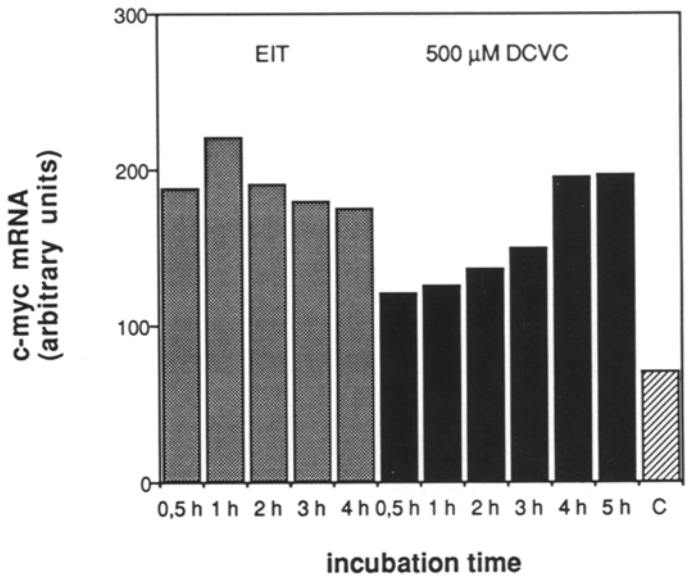

FIGURE 3. Effect of 20 ng/ml epidermal growth factor/20 mg/ml insulin/4 mg/ml transferrin (EIT) and 500 mM DCVC on the expression of *c-myc* in LLC-PK_1 cells; c = control, 1 hr incubation in DMEM/10% FCS after 24 hr serum deprivation (DMEM/0.2% FCS). The arbitrary units were obtained by densitometric scanning of the *c-myc* autoradiogram of Figure 1.

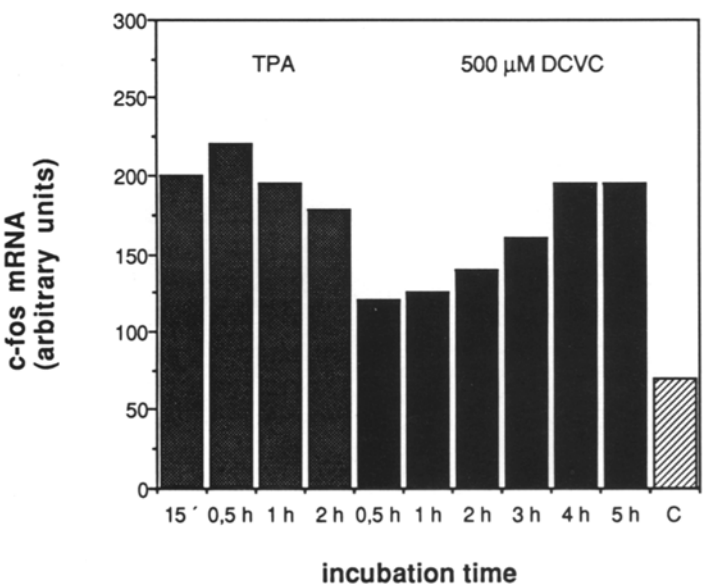

FIGURE 4. Effect of 300 nM TPA and 500 mM DCVC on the expression of *c-fos* in LLC-PK_1 cells, c = control, 0.5 hr incubation in DMEM/10% FCS after 24 hr serum deprivation (DMEM/0.2% FCS). The arbitrary units were obtained by densitometric scanning of the *c-fos* autoradiogram of Figure 4.

Incubation of LLC-PK$_1$ cells with 200 μM DCVC also induced *c-fos* and *c-myc* expression. The maximum levels of the transcripts by densitometric analysis amounted to approximately 70% of the values induced by 500 μM DCVC.

To determine if the DCVC-induced gene expression persists after removal of the compound, LLC-PK$_1$ cells were pulse-treated with 500 μM DCVC for 5 hr and incubated in DCVC-free medium for 24, 48 and 72 hr. Northern blot analysis of the RNA isolated at these time points did not reveal detectable *c-fos* and *c-myc* transcripts. In contrast to the disappearance of the DCVC effects on gene expression, the 5 hr pulse treatment induced a significant impairment of cell proliferation after 48 hr and 72 hr incubation in DCVC free medium (Figure 5).

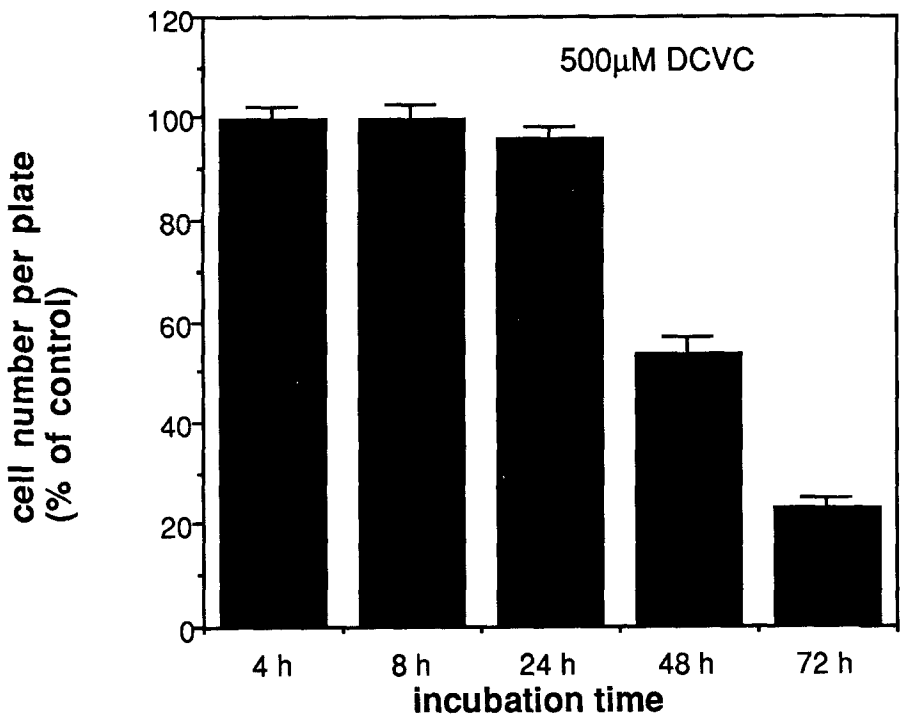

FIGURE 5. Effect of 500 mM DCVC on cell proliferation in LLC-PK$_1$ monolayers. Cells were pulse-treated with DCVC for 5 hr and the cell numbers were determined after incubation in DCVC-free medium at the times indicated. Results are mean ± SD of 9 wells from 3 independent experiments and are expressed in % of the cell numbers obtained from the untreated control.

DISCUSSION

S-(1,2-dichlorovinyl)-L-cysteine (DCVC) is the key metabolite responsible for the nephrotoxicity and nephrocarcinogenicity of trichloroethene and dichloroacetylene. DCVC is nephrotoxic in rats and mice and promotes dimethylnitrosamine-initiated kidney tumors in mice (Meadows et al., 1988; for a review see Dekant et al., 1989). DCVC is mutagenic in bacteria and induces DNA repair in the cultured kidney cell line LLC-PK$_1$. In addition to these

direct genotoxic effects, DCVC also induces activation of Ca^{2+}-dependent endonucleases, which resulted in increased formation of DNA double-strand breaks, followed by increased poly (ADP-ribosyl)ation of nuclear proteins. Hence, DCVC may influence gene expression, either by formation of DNA adducts or by the induction of Ca^{2+}-dependent DNA double-strand breaks, or by altering the function of nuclear proteins by increased poly(ADP-ribosyl)ation, which is one of the most effective posttranslational protein modifications (for a recent review see Vamvakas et al., 1992b). However, the demonstrated genotoxic effects of DCVC do not necessarily imply that modulation of gene expression also takes place. Since only a small part of the genomic DNA is transcriptionally active, many genotoxic events do not induce changes in gene expression. In addition, the nucleus has very efficient DNA-repair systems and is able to remove most of the DNA damage before it can be transformed into heritable mutations. Finally, depending upon the chemical nature of the DNA modification and the extent of the DNA damage, interactions of xenobiotics with DNA may result in cell death.

Relevant for tumor formation are the genetic events that are compatible with cell life and at the same time modulate gene expression, thus resulting in alterations of cell proliferation and/or differentiation. The present work investigates the effects of the nephrotoxin and possible nephrocarcinogen DCVC on the expression of *c-myc* and *c-fos* in cultured kidney cells (LLC-PK_1).

C-fos and *c-myc* play an important role in cell proliferation and differentiation, and may also contribute to malignant transformation (Stone et al., 1987; Littlewood and Evan, 1990; Angel and Karin, 1992). Deprivation of mitogenic stimuli induces a drastic reduction of the expression of *c-fos* and *c-myc* mRNA and results in arrest of the cells at the G_0 interphase (Moore et al., 1986). In agreement with the existing literature, the *c-fos* and *c-myc* transcripts were not detectable in LLC-PK_1 cells deprived of serum for 24 hr. Stimulation of these deprived cells with 10% FCS induced the expression of both oncogenes. The amount of the transcripts induced by serum stimulation was approximately the same at all sampling times (between 0.5 hr and 5 hr). Presence of DCVC in the high-serum incubation medium resulted in a further increase of the *c-fos* and *c-myc* expression; the maximum levels of the transcripts were approximately three-fold higher, compared to the values obtained from the cells that were stimulated only with serum. The DCVC-induced transcription was time-dependent, increasing continuously from 0.5 hr to 4 hr. In agreement with observations in several other cell lines (Müller et al., 1984), the *c-fos* transcription preceded the *c-myc* expression in LLC-PK_1 cells.

Growth factors such as EGF, transferrin and insulin induce the expression of *c-myc* in several experimental systems (Müller et al., 1984; Dean et al., 1986; Ran et al., 1987). The tumor promotor 12-*O*-tetradecanoylphorbolacetate (TPA) is a potent activator of the *c-fos* expression (Angel et al., 1987). Hence, in the present study a mixture of EGF, transferrin, and insulin (EIT) and TPA were used as positive controls for *c-myc* and *c-fos*, respectively. EIT induced a marked increase of the *c-myc* transcript levels in LLC-PK_1 cells, compared with the amounts observed in cells stimulated only with serum. TPA elicited a similar increase of the *c-fos*

expression. The maximum levels of the transcripts by densitometric analysis were similar to the levels induced by DCVC. However, the time-pattern of the expression of the two protooncogenes in the presence of growth factors or TPA was clearly different from the time-dependent increase observed with DCVC. In the presence of TPA, the maximum levels of the *c-fos* transcript were obtained 0.5 hr after addition of the compound. Densitometric analysis of the autoradiogram bands in the presence of EIT revealed highest values 1 hr after addition of the growth factor mixture. In both cases, the oncogene transcripts decreased slightly up to the 4 hr sampling time. In contrast, DCVC elicited a consistent, time-dependent increase of the transcription of the two oncogenes in the 5 hr incubation time. The time-pattern obtained with the growth factors and with TPA indicates regulation of the expression of *c-fos* and *c-myc*, either by the transcripts themselves or by induction of pathways repressing gene expression. This is in agreement with several previous studies showing that, in normal non-transformed cells stimulated to enter the cell cycle, the expression of *c-fos* and probably also of *c-myc* is transient and underlies strict regulating mechanisms. These mechanisms are often impaired in transformed cells which exhibit, in many cases, a permanent increased expression of *c-fos* and *c-myc* (Campisi et al., 1984). The independence of protooncogene expression upon external and intracellular regulating mechanisms contributes to the uncontrolled cell proliferation of transformed cells.

Growth factors and TPA induce gene expression by activation of physiological signal transduction pathways involved in cell proliferation. The striking differences in the time-pattern between these inducers and DCVC, and also the fact that DCVC did not influence cell proliferation at the concentrations used in the present study, indicate that other mechanisms may be involved. As described above, DCVC may induce both base mutations and also Ca^{2+}-mediated DNA double-strand breaks and increased poly(ADP-ribosyl)ation of nuclear proteins. If the induction of gene expression results from the formation of DNA-adducts that are converted into heritable mutations, one would expect the effects to persist after removal of DCVC. However, the *c-myc* and *c-fos* transcripts could not be detected when the LLC-PK_1 cells were pulse-treated with DCVC for 5 hr and then incubated in DCVC-free medium for 24, 48 and 72 hr, providing indirect evidence that induction of DNA-double strand breaks and increased poly(ADP-ribosyl)ation of nuclear proteins by perturbation of the intracellular Ca^{2+} homeostasis may be more important in the DCVC-induced expression of *c-fos* and *c-myc*. Ca^{2+} is a cell messenger that seems to play an important role in the control of cell cycle and gene expression, both in physiological and toxic conditions (Whitaker and Patel, 1990). Although direct evidence is still lacking, Ca^{2+} seems to also be involved in malignant transformation and tumor formation. The Ca^{2+} ionophore A23187 induces *c-fos* mRNA accumulation with similar kinetics as the tumor-promoting phorbol esters in human monocyte-like cells (Shibanuma et al., 1987). Furthermore, the tumor promotor thapsigargin, which increases the cytosolic Ca^{2+} concentrations by inhibiting the Ca^{2+}-ATPase of the endoplasmic reticulum, also induces *c-fos* expression (Schönthal et al., 1991). Finally, Ca^{2+} plays an important role in the induction of *c-fos* expression in primary cultures of proximal tubule cells exposed to oxidative stress generated by xanthine/xanthine oxidase (Maki et al.,

1992). In this study, chelation of intracellular Ca^{2+} by Quin-2, or of extracellular Ca^{2+} by EGTA, drastically reduced the *c-fos* expression.

In summary, treatment of LLC-PK1 cells with DCVC induces a time-dependent expression of the protooncogenes *c-fos* and *c-myc*. The demonstrated genomic modulation may be involved in the induction of renal cell carcinomas observed with the parent compounds trichloroethene and dichloroacetylene in the rat.

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