Effect of Diquat on Cell Growth and Macromolecule Synthesis in Cultured Pneumocytes

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SAITO, K. *Effects of Diquat on Cell Growth and Macromolecule Synthesis in Cultured Pneumocytes.* Tohoku J. exp. Med., 1986, **148** (1), 35-39 — The effects of diquat (DQ) on cell growth and macromolecule synthesis were investigated in cultured rat, feline and human. pneumocytes. DQ at 10^{-3} M and 10^{-5} M showed time- and dose-dependent inhibition of cell growth for 7 days. The incorporation of ^3H -thymidine, ^3H -uridine and ^{14}C -leucine into DNA, RNA and protein was reduced to 0-47% by 10^{-3} M of DQ, and that of ^{14}C -leucine into protein of A-549 cells was reduced to 67% by 10^{-5} M of DQ. Among the cells examined, A-549 cells derived from human lung were the most resistant to the inhibitory effects of DQ on cell growth and macromolecule synthesis. On the other hand, human lung embryonic fibroblast cells and L-2 cells derived from rat lung were the most sensitive to the toxicity of DQ. These results indicate that the inhibitory effects of DQ on cell growth and on macromolecule synthesis are dependent on the concentrations of DQ administered, and that species differences in the sensitivity to DQ toxicity may exist. ——— diquat; pneumocytes; DNA; RNA; protein

Diquat (N, N'-ethylene-2, 2' bipyridilium, DQ), which is a bipyridilium salt, has been shown to have herbicidal property. Its herbicidal activity appears to depend, in part, on the ease of one electron reduction of DQ^{++} to form superoxide anion (O_2^-) from the stable, but air-sensitive radical cation of DQ^+ (Dodge 1971). It has been suggested that DQ injected intravenously into mice is distributed throughout most tissues, except for brain and spinal cord (Litchfield et al. 1973). Animals given DQ die without obvious histological changes that might account for death (Clark and Hurst 1970).

Recently, an accidental lethal poisoning by DQ ingestion was reported (Powell et al. 1983). DQ has been said to affect cyclic AMP, ACTH or glucose level of rat (Rose et al. 1974), but the biological effects underlying the mammalian toxicity of DQ are not fully understood. Once DQ has been suggested to be accumulated into cultured pneumocytes (Saito and Menzel 1979), and the cells used in the present study have been shown to be sensitive to the toxicity of paraquat (PQ), another bipyridilium herbicide related to DQ (Saito 1985). This

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study of the biochemical effects occurring in cultured type II pneumocytes of three species after DQ administration has been undertaken in an attempt to understand the metabolic disturbances underlying the toxicity of DQ.

MATERIALS AND METHODS

Cell culture. Four kinds of cell lines, AK-D cells derived from feline embryonic lung (Kniazeff et al. 1976), L-2 cells derived from rat lung (Douglas and Kaighn 1974), A-549 cells derived from human lung (Lieber et al. 1976) and human embryonic lung fibroblast cells (HEL) (Peterson et al. 1978), were obtained from American Type Culture Collection (Rockville, MD, USA). AK-D cells and L-2 cells were cultured in 3 ml of F-12K culture medium containing 15% and 20% fetal calf serum (FCS), respectively, A-549 cells and HEL cells were cultured in 3 ml of Dulbecco's modified minimum essential medium with Earle's salts containing 10% FCS in 25 cm² plastic tissue culture flasks at 37°C. The tissue culture media were supplemented with 100 units/ml of penicillin G and 100 µg/ml of streptomycin.

Effect of DQ on cell growth. AK-D cells, L-2 cells and A-549 cells were cultured with 10⁻⁵ M and 10⁻³ M of DQ or PQ for 7 days at 37°C. The primary cell number was adjusted to 5 × 10⁴ cells/ml. At each 24 hr, cells were trypsinized with 0.125% of trypsin and 0.02% of ethylenediamine tetraacetic acid (EDTA) solution and cell numbers were counted in a hemocytometer. Tissue culture media were changed at each 2, 4 and 6 days in culture. DQ and PQ were also added to the new culture media.

Effect of DQ on macromolecule synthesis. Cells were incubated with 10⁻³ M and 10⁻⁵ M of DQ at 37°C for 24 hr. At the beginning of incubation, 0.05 µCi/ml of ³H-thymidine (specific activity; 2 Ci/mmol, ³H-TdR), ³H-uridine (specific activity; 2 Ci/mmol, ³H-UdR) and ¹⁴C-leucine (specific activity; 50 mCi/mmol, ¹⁴C-LeR) were added to the media. The incubation was terminated by washing the cells with ice cold Dulbecco's phosphate buffered saline solution (PBS), pH 7.4, for three times. The cells were treated with ice cold 10% trichloroacetic acid (TCA) for 10 min and then 5% TCA for 20 min twice and were dehydrated with addition of absolute ethanol at room temperature for 20 min. The cells were dissolved by addition of 1 N NaOH. Aliquots were used to measure protein concentration by the method of Lowry (Lowry et al. 1951), other aliquots were neutralized with 1 N HCl in order to count the radioactivity in POPOP liquid scintillation cocktail by a liquid scintillation technique. The cell protein concentration was standardized by calf serum albumin. The cellular radioactivity was expressed to count per minute (cpm)/mg protein. The static analysis of the data was performed by Student's *t* test. Tissue culture materials were obtained from GIBCO (Gland Island, NY, USA). The radioactive precursors were obtained from New England Nuclear (Boston, MA, USA). Other chemical reagents were obtained from Sigma Chem. Co. (St. Louis, MO, USA).

RESULTS

Cell growth. DQ produced a time- and dose-dependent inhibition of cell growth. DQ at 10⁻³ M and 10⁻⁵ M had significantly ($p < 0.001$ at 10⁻³ M and $p < 0.01$ at 10⁻⁵ M) stronger inhibitory effect on the cell growth, than did PQ at 10⁻³ M and 10⁻⁵ M. After 48 hr in culture with 10⁻³ M of DQ, appreciable cell numbers in the flasks were not detected with AK-D cells (Fig. 1). In the other two cell lines, L-2 cells and A-549 cells, DQ at 10⁻³ M and 10⁻⁵ M had similar inhibitory effect on cell growth (Data not shown).

Macromolecule synthesis. The incorporation of radioactive precursors of ³H-TdR, ³H-UdR and ¹⁴C-LeR into acid insoluble fractions of DNA, RNA and

Fig. 1. Effect of DQ and PQ on cell growth of AK-D cell.

Each point indicates mean numbers of cells/ml of three samples.

Culture condition is presented in the text. ○—○, control; △—△, DQ (10^{-5} M); ▲—▲, DQ (10^{-3} M); □—□, PQ (10^{-5} M); ■—■, PQ (10^{-3}); ↑, changing the culture medium.

* $p < 0.05$; ** $p < 0.01$.

protein, respectively, was significantly inhibited by 10^{-3} M of DQ for 24 hr in all of cells examined. The incorporation of ^3H -TdR into DNA was reduced to 0% in L-2 cells and 24% in A-549 cells that of ^3H -UdR into RNA was reduced to 0% in L-2 cells to 47% in A-549 cells and that of ^{14}C -LcR into protein was also reduced to 0% in HEL cells and to 45% in A-549 cells by 10^{-3} M of DQ for 24 hr in culture. In the four cell lines examined, L-2 cells and HEL cells seemed to be most sensitive to DQ toxicity, and A-549 cells were most resistant to DQ toxicity. At a concentration of 10^{-5} M, DQ had no inhibitory effect on the incorporation of these precursors into macromolecules for 24 hr, except the reduction of ^{14}C -LcR incorporation into protein of A-549 cells to 68% (Table 1). The total protein concentration of the cells in culture were reduced to 37% in AK-D cells, 65% in L-2 cells, 70% in HEL cells and 28% in A-549 cells. Thus a species difference in the ability of DQ to cause toxicity seemed to exist.

DISCUSSION

In the present study, the biochemical effects of DQ on cultured pulmonary cell lines were examined to evaluate the toxicity of DQ. Because the same cell type could be derived from different species and the ease of propagation of cells

TABLE 1. *Effect of diquat on ^3H -TdR, ^3H -UdR and ^{14}C -LcR incorporation into the cells for 24 hr*

Cells		^3H -TdR	^3H -UdR	^{14}C -LcR
L-2 (n=4)	Control	297.4 $\pm$ 17.4	418.5 $\pm$ 55.5	279.5 $\pm$ 66.4
	DQ (10 ⁻³ M)	0	0	8.8 $\pm$ 1.7**
	DQ (10 ⁻⁵ M)	267.1 $\pm$ 17.9	456.3 $\pm$ 37.7	264.6 $\pm$ 38.3
AK-D (n=4)	Control	113.9 $\pm$ 14.0	101.1 $\pm$ 3.2	134.4 $\pm$ 8.4
	DQ (10 ⁻³ M)	15.2 $\pm$ 5.6**	42.4 $\pm$ 1.3**	19.2 $\pm$ 4.7**
	DQ (10 ⁻⁵ M)	97.5 $\pm$ 4.4	102.1 $\pm$ 4.8	128.2 $\pm$ 11.1
A-549 (n=4)	Control	743.6 $\pm$ 63.6	278.1 $\pm$ 11.9	209.7 $\pm$ 8.6
	DQ (10 ⁻³ M)	177.9 $\pm$ 14.9**	132.4 $\pm$ 35.7**	94.6 $\pm$ 6.4**
	DQ (10 ⁻⁵ M)	690.8 $\pm$ 58.9	271.9 $\pm$ 25.0	142.5 $\pm$ 16.8*
HEL (n=3)	Control	209.0 $\pm$ 47.4	160.6 $\pm$ 1.8	59.9 $\pm$ 5.4
	DQ (10 ⁻³ M)	2.0 $\pm$ 0.3**	2.1 $\pm$ 1.1**	0
	DQ (10 ⁻⁵ M)	197.7 $\pm$ 38.2	161.1 $\pm$ 6.1	54.1 $\pm$ 5.4

Asterisks indirate significant (* p < 0.05; ** p < 0.01) difference of the control.

Values are means $\pm$ s.d. of cpm ($\times 10^2$)/mg protein.

Culture condition is presented in the text.

in culture, it was decided to use the four cell lines derived from three species. It has been reported that PQ has an inhibitory effect on macromolecule synthesis in these cell lines, i.e., the incorporation of ^3H -TdR into DNA, that of ^3H -UdR into RNA and that of ^{14}C -LcR into protein were all eliminated to approx. 50% by 10⁻³ M of PQ (Saito 1985). In this experiment, DQ had more marked inhibitory effect on cell growth and on macromolecule synthesis than PQ. The difference of the efficacy of these bipyridilium salts as inhibitors may be related to their redox potential, i.e., E_0 ; -348 mV for DQ and -446 mV for PQ.

It has been suggested that DQ at 10⁻³ M does not alter the incorporation of ^{14}C -LcR into protein of P 338D1 "Macrophage-like" cell line (Carminc et al. 1981). In the present study, DQ inhibited protein synthesis by 55%-100% in all kinds of cell lines examined, and by 32% at 10⁻⁵ M in A-549 cells. The difference between P 338D1 cells and the four cells examined in response to DQ toxicity is not clear.

A-549 cells was shown to have a much higher ability to induce the level of superoxide dismutase by PQ than L-2 cells (Saito 1985). The more marked inhibitory effect of DQ at 10⁻³ M on DNA, RNA and protein synthesis in L-2 cells and HEL cells than that in A-549 cells and AK-D cells may be due to the lower induction of superoxide dismutase in L-2 cells and HEL cells by DQ relative to A-549 cells and AK-D cells.

The biochemical mechanisms of the lethality of DQ is not clearly understood. It is presumed that DQ produces metabolic disturbances, such as an increase of glucose utelization in the liver of that of cyclic AMP in the adrenal gland of the

rat (Rose et al. 1974) and depletion of NADPH in the lung of the rat (Witschi et al. 1977). In the present study, it was demonstrated that DQ has an inhibitory effect on cell growth. The inhibition of the cell growth might be related to the inhibition of incorporation of precursors into DNA, RNA and protein in the cells. The inhibition of uptake of these precursors into the cells by high concentration of DQ might be involved in the acute toxic mechanisms of DQ in humans and animals.

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