ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 49, nr 4: 357—361
1980

Influence of cyclophosphamide on respiration and membrane permeability of plant cells

MARIA PODBIELKOWSKA, ALICJA ZOBEL, MARIA WAŁĘZA

Department of General Botany, Institute of Botany, Warsaw University, ul. Krakowskie Przedmieście 26/28, 00-325 Warszawa, Poland

(Received: December 3, 1979)

Abstract

A specific influence of cyclophosphamide, an oncostatic drug of the group of alkylating agents, has been demonstrated on cellular respiration and the permeability of cell membranes. The tested drug under the experimental conditions inhibites cell respiration by about 20-30 per cent as compared with the control. The permeability of the plasmalemma and tonoplast decreased markedly under the action of cyclophosphamide.

INTRODUCTION

It has been demonstrated in an earlier paper (Podbielkowska, Nowaczek, 1979) that cyclophosphamide, an oncostatic drug of the group of alkylating agents (Boesen, Davis, 1972) causes a characteristic membranisation of the protoplast in meristematic cells of aerobically respirating roots.

Since a similar reorganisation of the protoplast was observed by some authors under the action of respiratory inhibitors (David, 1970; Podbielkowska et al., 1975), or when energetic deficit was induced in various ways in the cell (Podbielkowska, Kupidłowska, 1976), the supposition was advanced that cyclophosphamide may influence the metabolism of high-energy compounds in the cell.

The experiment described below was carried out to test the influence of this drug on the process of cellular respiration and the permeability of the cell membrane.

MATERIAL AND METHODS

The experiment was carried out with adventitious roots of *Allium cepa* L. grown in hydroponic culture in vessels of 250 ml volume in tap

water changed every 24 h. When the roots reached 2-3 cm they were transferred with the bulbs to 0.4 per cent cyclophosphamide (Germed) solution for 6, 12 and 24h.

The concentration of the tested oncostatic was established on the basis of the effectiveness of the doses (Podlewski, Chwalibogowska-Podlewska, 1978) and the previously performed experiments (Podbielkowska, Nowaczek, 1979).

As control material served roots growing in tap water.

The respiration rate was investigated in a Warburg apparatus (Dixon, 1951) by measuring oxygen uptake. The medium consisted of the cytostatic drug. One-centimetre root samples used for the measurements were of equal weight. Uptake of O_2 (µl/g fresh mass) was determined at 10-min intervals over 2 h. The results of oxygen uptake were compared with the control ones, and the differences in respiration intensity are given as per cent of the control value. Three experimental combinations were tested, each with 12 samples.

To demonstrate if there is any relation between the degree of permeability of the plasmalemma and tonoplast and the action of the drug, the deplasmolysis rate was measured in an experiment. Fragments of the upper epidermis of the onion leaf were incubated for 60 min in an 0.4 per cent cyclophosphamide solution and plasmolysed in a 30 per cent sucrose solution, and then the deplasmolysis rate was determined in 0.6 M ethyl glycol which penetrates relatively slow into the cells. The time was measured from the moment of placing the tissue in the deplasmolyser solution to the end of complete deplasmolysis of all cells in the field of vision (ca. 20 cells) for each of the 10 tests.

RESULTS

The results of respiration intensity measurement are shown in Table 1 giving mean values from 36 tests in three series of experiments.

Table 1

Influence of cyclophosphamide on respiration of onion root growth apexes

Time of exposure to drug, h	Respiration in per cent of control			
	I comb.	II comb.	III comb.	mean from 3 combinations
6	70.0	67.2	77.5	71.6
12	73.7	83.8	72.3	76.9
24	77.7	73.7	80.9	77.4

It results from the data obtained that the cellular respiration rate was reduced by the action of 0.4 per cent cyclophosphamide to 70-80

Table 2

Influence of cyclophosphamide on permeability of plasmalemma and tonoplast in epidermal cells from onion scales

Deplasmolysis time min.	Mean number of deplasmolyzed cells in successive minutes of experiment	
	cyclophos-phamide	distilled water
3	—	1
6	—	2
9	—	4
12	2	12
15	8	20
18	9	
21	11	
24	13	
27	16	
30	20	

per cent of that in the control, equally after 6, 12, and 24 h of action of drug. The effect was constant during the 2 h when the experiment was run in all three experimental combinations.

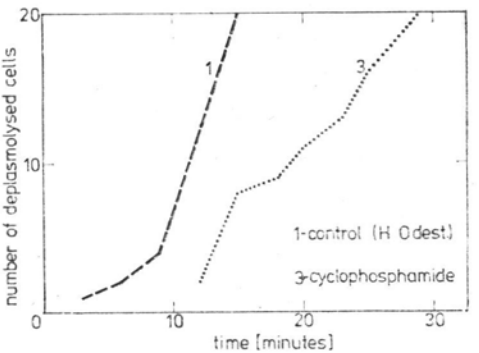

Fig. 1. Mean number of deplasmolyzed cells in the successive minutes of the experiment

The metabolic processes connected with energy production are closely related with active transport and the permeability of the cell membranes. Therefore, as the next step of the experiment the permeability of the tonoplast and plasmalemma was measured by the plasmolytic method. The data are shown in Table 2 and in Fig. 1. As seen, there is a distinct relation between the degree of permeability of the plasmalemma and tonoplast and the action of the cytostatic. The membrane permeability is reduced under the action of cyclophosphamide.

DISCUSSION

The results of the experiment show a specific action of cyclophosphamide on cellular respiration and membrane permeability. The preparation tested has an action analogous to respiratory inhibitors, manifested by a decrease in the intensity of aerobic respiration. This is associated with a characteristic reorganisation of the ergastoplasm, appearing in hypertrophy of the rough endoplasmic reticulum (rER) membranes and an increase in the number of mitochondria and ribosomes (Podbielkowska, Nowaczek, 1979).

Similar changes in the protoplast structure were observed in cells under conditions of energetic deficit (Podbielkowska, Kupidłowska, 1976). In such conditions the factor stimulating glycolysis is intensively synthesised (Nejfach, 1973). It has also been demonstrated that glycolysis stimulation is associated with the development of ER membranes (Podbielkowska, Kupidłowska, 1976). This fact prompted the hypothesis that ER membrane hypertrophy occurs in order to increase the active surface in glycolytic processes. Glycolysis stimulation is not, however, the only factor inducing the above described protoplast reorganisation. There also exists a distinct connection between membranisation of the cytoplasm and disturbances in the respiratory chain (Podbielkowska et al., 1975). This observation agrees with the suggestion of Gordon et al. (1975) who found within the ER a reaction of the respiratory chain with a different enzymatic composition than that in the mitochondria.

Cyclophosphamide tested in the present study may thus be a specific factor disturbing cellular respiration by acting on the mitochondrial and cytoplasmic electron transport chain. The presently observed changes in the permeability of the plasmalemma and tonoplast, due to the action of cyclophosphamide, suggests that under conditions of energetic deficit there also occur transport disturbances in the endoplasmic membranes.

REFERENCES

Boesen E., Davis W., 1972. Chemioterapia nowotworów. PZWL. Warszawa.

David H., 1970. Zellschädigung und Dysfunktion. Protoplasmatologia 10. Springer-Verlag, Wien-New York.

Dixon M., 1951. Manometric Methods, 3-rd ed. University Press Cambridge.

Gordon L. H., Alekseeva U. J., Bichurina A. A., 1975. The study of extramitochondrial oxydative systems in intact plant tissues. XII International Botanical Congres, Leningrad (abstracts).

Nejfach S. A., 1973. Mechanizmy integracji przemian komórkowych. PWN Warszawa.

Podbielkowska M., Żarska-Maciejewska B., Kacperska-Palacz A., 1975. Morphology of protoplast as affected an inhibition of respiration. Protoplasma 83: 469-474.

Podbielkowska M., Kupidłowska E., 1976. Changes in structure and function of plant cell protoplast due to energy deficit. Acta Soc. Bot. Pol. 45: 239-250.

Podbielkowska M., Nowaczek M., 1979. Effect of cyclophosphamide on meristematic plant cells. Acta Soc. Bot. Pol. 48: 355-363.

Podlewski J. K., Chwalibogowska-Podlewska A., 1978. Leki Współczesnej terapii. PZWL, Warszawa.

Działanie cyklofosfamidu na oddychanie komórkowe i przepuszczalność błon w komórkach roślinnych

Streszczenie

Badano skutki działania cyklofosfamidu, onkostatyku zaliczanego do grupy związków alkilujących, na oddychanie komórkowe i przepuszczalność błon w komórkach roślinnych. Stwierdzono, że cyklofosfamid hamuje oddychanie tlenowe w badanych tkankach o około 20-30% w stosunku do kontroli. Przepuszczalność plazmalemmy i tonoplastu pod wpływem cyklofosfamidu wyraźnie zmniejszyła się. Zahamowany proces oddychania tlenowego znajduje wyraz w typowej dla działania inhibitorów oddechowych reorganizacji ergastoplazmy. Wyraża się ona hypertrofią membran szorstkiego ER, a także wzrostem liczby rybosomów i mitochondriów (Podbielkowska, Nowaczek, 1979).