Mitotic activity, submicroscopic organization and respiration of meristematic cells of root tips of *Allium cepa* L. treated with dacarbazine (DTIC)

MARIA PODBIELKOWSKA, ANNA ŚLUBORSKA, MARIA WĄŁĘZA

Department of General Botany, Institute of Botany, University of Warsaw,
Krakowskie Przedmieście 26/28, 00-927 Warszawa, Poland

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Abstract

Studies were carried out on the effect of dacarbazine (DTIC) on meristematic cells of root tips of *Allium cepa* L. Noticeable mitodepression was observed. It depended upon time of exposure and DTIC concentration. Mitodepression was caused by an inhibition of DNA synthesis and blocking of the G₂ phase. DTIC caused also disturbances of the karyokinesis and cytokinesis, similar as in case of colchicine effect. In the experimental conditions inhibition of cell respiration was noted, as also changes in the tissue ultrastructure. These changes were typical of the effect of respiratory inhibitors.

INTRODUCTION

Dacarbazine (5-(3,3 dimethyl-1 Triazeno)-imidazol-4-carboxamide — DTIC) is a pharmacological compound, discovered in the seventies, and used in tumour therapy. It was applied with good results, both in mono- and complex therapy (Karrer 1978, Kühböck et al. 1978, Kokoschka 1978, Titscher 1978). Mechanisms of DTIC action are not fully known yet. There are three hypotheses of the action: 1) that DTIC inhibits DNA synthesis as purine analogue (antimetabolite), 2) that it acts as an alkylator, and 3) that it reacts with SH groups.

At the beginning dacarbazine is inactive. According to Karrer (1978) two mechanisms activate DTIC, depending on the conditions, i.e. 1) under the effect of light methyl radicals get free, and purine analogue is formed which becomes incorporated into nucleic acids (antimetabolite); 2) demethylation with the participation of microsomal oxydases (alkylator).

Due to the fact that mechanism of DTIC action is still controversial, studies have been undertaken on the effect of this substance upon meristematic tissue. Several studies already showed that plant material can be used for rapid and easy testing of mutagenic properties and of specificity effect of oncostatic upon cell cycle (Broda and Kocik 1972, Guzewska 1977, Moore 1978).

MATERIAL AND METHODS

Roots of onion (*Allium cepa* L.) were cultured in 250 ml jars filled with tap water. Water was changed every day. Roots 2-3 cm long were transferred together with onion bulbs into dacarbazine (DTIC) solutions (produced by Dome, USA), at concentrations of 500, 750 and 1000 ppm in tap water. Control material consisted of onion bulbs cultured in tap water only.

Mitotic activity of roots was determined after 3, 6, 12 and 24 hours of dacarbazine treatment. In order to check whether the changes were reversible, after 24 hours of DTIC treatment some onion bulbs were again transferred to tap water for 0.5, 1, 3, 6, 24 and 72 hours.

Growing points of the roots were fixed in acetoalcohol (volume ratio 1:3) for 2 hours. Fixed material was squashed in acetoorcein with 1 N HCl (volume ratio 9:1), or stained with Feulgen method. Mitotic activity of the meristem was determined taking into account all cell divisions in 1000 cells from 7 fields (López-Sáez and Fernandez-Gomez 1965), with attention paid to the number of particular mitotic phases. Squashed preparates were also used for determining changes of chromosomal structure, caused by DTIC.

Level of DNA and protein synthesis was determined autoradiographically, with the use of radioactive thymidine and leucine. Analyses were made of the incorporation of radioactive precursors into the roots treated for 24 hours with DTIC solutions of 500 ppm and 750 ppm. Precursor at concentration of 5 $\mu\text{Ci/ml}$ was introduced in the last hour of the incubation. For testing reversibility of changes, after 24 h of DTIC treatment (at concentrations of 500 and 750 ppm) roots were transferred to tap water for 24 and 72-hour postincubation. In the last hour of postincubation H^3 -thymidine and H^3 -leucine were introduced at concentration of 5 $\mu\text{Ci/ml}$. Roots growing in water, transferred to H^3 -thymidine and H^3 -leucine similarly as those treated with DTIC, served as the control.

Material was fixed in acetoalcohol. Preparations were made squashing the material in acetoorcein. Preparations were then covered with photographic emulsion AR 10 Kodak for 14 days.

Table 1

Mitotic and phase index (in %) in the tip meristem of onion (*Allium cepa* L.) roots treated with DTIC compare to the control

Time in hours	Incubation																						
	control				DTIC 500 ppm				DTIC 750 ppm				DTIC 1000 ppm										
	3	6	12	24	3	6	12	24	3	6	12	24	3	6	12	24							
Mitoses in 1000 cells	6.60	7.33	6.26	6.55	6.32	5.03	4.13	3.30	—	5.66	—	3.73	5.56	4.50	1.86	1.03							
Prophases	34.6	32.0	34.6	35.1	31.7	39.5	39.1	39.5	—	34.3	—	36.5	33.0	34.3	36.8	38.5							
Metaphases	22.5	24.0	22.8	22.7	28.2	22.1	22.1	22.0	—	30.0	—	24.3	31.0	24.5	26.3	23.5							
Anaphases	19.6	22.5	24.1	20.8	21.1	18.6	19.0	19.3	—	18.0	—	20.2	18.2	20.3	16.8	18.9							
Telophases	23.1	21.5	18.3	21.2	18.8	19.6	19.6	19.1	—	17.6	—	19.0	17.8	20.8	28.6	19.0							
Time in hours	postincubation after 24 h of incubation																						
	0.5	1	3	6	24	72	0.5	1	3	6	24	72	0.5	1	3	6	24	72	0.5	1	3	6	24
Mitoses in 1000 cells	6.60	6.65	6.80	7.15	7.16	8.96	5.10	7.50	6.35	7.40	7.33	8.66	5.05	6.70	5.70	6.40	6.13	7.50	dead cells during whole postincubation				
Prophases	37.7	34.7	35.2	34.7	32.3	28.6	58.0	45.2	49.2	46.5	30.8	32.1	58.5	46.6	47.5	44.7	24.6	37.5					
Metaphases	23.7	23.7	21.7	23.5	20.6	27.1	18.0	27.7	19.7	23.7	30.6	23.3	17.5	26.5	22.5	25.5	15.5	28.5					
Anaphases	20.0	19.2	19.7	20.0	22.1	21.3	11.7	14.0	12.5	14.5	19.1	21.8	11.0	13.5	13.5	14.5	13.8	16.5					
Telophases	19.2	19.7	19.0	21.5	24.8	21.8	12.2	13.0	18.5	15.2	19.3	22.6	13.0	13.5	16.5	15.2	13.8	17.5					

In order to analyse disturbances of the mitosis course, root tips were fixed in chrom-aceto-formalin, at percentage ratio 0.5:1:20. Preparations 4-5 μm thick (prepared with paraffin method) were stained with Heidenhain hematoxylin and toluidine blue, at pH 3.4 and 7.4.

Electron microscopy was performed on 2 mm fragments of top parts of the roots, from which root-caps were removed. 2% KMnO_4 or glutaraldehyde were used to fix the material (Karnovsky 1965). Roots were immersed in epon; scraps were contrasted with Reynolds' method (1963). Observations were made on BS 500 (Tesla) microscope.

In the second stage of the experiment, analyses were made of the effect of DTIC upon respiratory processes. Roots were treated (similarly as in the first stage) with DTIC, at concentrations of 500 and 750 ppm for 24 h. Level of respiration was determined in Warburg apparatus, measuring intensity of oxygen consumption (Dixon 1951). Almost the same mass of 1 cm long root tips was placed in pots, in cyto-static solutions. Consumption of $\text{O}_2/\text{ml/g}$ fresh mass was measured at 10 min intervals for 2 hours. Ten samples were made for each DTIC concentration, together with 10 controls.

RESULTS

DTIC is characterized by noticeable mitodepression effect. It was reflected by a decrease of mitotic index in the incubated material (Table 1). Decrease of the index depended on the DTIC concentration and duration of oncostatic action. The strongest effect was observed after treatment with DTIC, at concentration of 1000 ppm. Already after 24 hours of this treatment the changes were irreversible. Decrease of mitotic index was observed also as a result of DTIC treatment at concentrations of 500 and 750 ppm, and mitostatic effects differed insignificantly (Table 1).

Analysis of the mitotic index showed that relative frequency of mitosis phases after DTIC treatment differed from the control. DTIC at all concentrations resulted in an increase of the number of prophases (Table 1). Also number of metaphases increased, especially at higher cytostatic concentrations (750 and 1000 ppm). Number of anaphases and telophases was lower than in the control at all concentrations.

Mitostatic effects of DTIC treatment were largely reversible. During postincubation in water visible increase of the mitotic index was noted (Table 1). After 24 h postincubation it reached values higher than in the control, whereas after 72 hours it reached values characteristic for the control material (Table 1). Relative frequency of particular mitotic phases was similar as in the control.

In roots treated for 24 hours with DTIC (at concentrations of 500 and 750 ppm) and then transferred to water for 0.5 hour, there was a noticeable unblocking of cell divisions (Table 1). During 1 hour post-incubation, mitotic index reached value close to the index characteristic of 24 hour postincubation (Table 1). Visible differences were also noted in relative frequency of the phases. Extremely large number of pro-phases was noted, especially after 0.5 h postincubation, at simultaneously low number of other phases (Table 1). During 1 h postincubation number of metaphases increased, whereas number of prophases slightly decreased. These data suggest that DTIC causes blocking of the cell mitotic cycle in the G_2 phase.

The effect of DTIC on synthesis of proteins was studied with the use of autoradiography. Preliminary analysis showed that DTIC inhibited DNA synthesis, and only slightly synthesis of proteins (Figs. 17, 18, 20, 21). In roots transferred to water for 24 hours, both DNA and protein synthesis increased (Figs. 19 and 22).

DTIC disturbed also the course of cell divisions. Characteristically loose prophases were observed, with visible internal structure of the chromosomes (Fig. 1). Metaphase chromosomes were strongly shorter and showed no equator orientation (Fig. 4). Such pattern of chromosomes can suggest changes in the organization of karyokinesis spindle, especially as chromosomal threads seem to form parallel patterns (Fig. 11). Chromosomes shaped as x-es and pliers (Fig. 4) were probably the effect of delayed kinetochore division.

In the anaphases (Figs. 3, 5, 7) unsimultaneous separations of chromosomes were observed and diminution of fragments or whole chromosomes from the plate. The lowest DTIC concentration induced characteristic bridges, which could have resulted from increased viscosity of the chromosomes (Fig. 12). After treatment with higher DTIC concentrations, anaphases were observed in which chromosomes, divided into chromatids, formed characteristic ski-like forms scattered throughout the whole cell (Fig. 2). It seems that such pattern results from a destruction of karyokinetic spindle. Division of chromosomes into chromatids showed, on the other hand, that kinetochor was only slightly damaged. Occurance of multipolar anaphases (Fig. 10) was probably caused by some damage of long threads of the spindle. C-anaphases were also observed, in which telophase transformations formed restitutional nuclei (Fig. 9). After treatment with lower DTIC concentrations characteristic chromatid bridges were observed in the telophases, with loose chromatin (Fig. 8). "Lost" chromatids were also present, from which micronuclei were formed (Fig. 7). C-anaphases induced by the highest DTIC concentration resulted in C-telophases (Fig. 13).

Fig. 1. Prophase, loose pattern of chromosomes. DTIC 500 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 2. C-Anaphase, chromosomes in a ski-pattern. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 3. Metaphase and late Anaphase, loose chromosome structure. DTIC 750 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 4. C-Metaphase, chromosomes significantly shortened. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 X

Fig. 5. Anaphase chromosomes, no polar orientation. DTIC 750 ppm, incubation — 24 h, acetoorcein, 2500 X

Fig. 6. Permanent joining between sister nuclei, partial primary wall. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 X

Fig. 7. Elimination of chromosomes or their parts. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 X

Fig. 8. Chromatid bridges. DTIC 500 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 9. Anaphase chromosomes from restitutional nuclei. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 10. Multipolar anaphase with chromatid bridges. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 11. Parallel pattern of chromosome threads. DTIC 500 ppm, incubation — 24 h, CrAF, iron hematoxylin, 2500 $\times$

Fig. 12. Chromatid bridges. DTIC 500 ppm, incubation — 24 h, CrAF, iron hematoxylin, 2500 $\times$

13

15

14

16

Fig. 13. Restitutional nucleus. DTIC 1000 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 14. Tri-nuclear cell, partial primary wall. DTIC 750 ppm, incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 15. Uneven distribution of chromatin, forming bridges. DTIC 1000 ppm incubation — 24 h, acetoorcein, 2500 $\times$

Fig. 16. Symmetrically arranged two micronuclei formed from eliminated chromosome parts. DTIC 750 ppm, incubation — 24 h, postincubation — 24 h, acetoorcein, 2500 $\times$

Chromatid bridges (Fig. 15) reflected disturbances of cytokinesis process, induced by DTIC treatment, and so did partial primary walls (Fig. 6), fusion of telophase nuclei (Fig. 15), and two- or three-nuclei cells (Fig. 14). Dacarbazine at concentration of 500 and 750 ppm did not have prolonged effect. Cell divisions commenced during postincubation and were quite normal. Elimination of chromatids and chromosomes from the mitotic apparatus caused in micronuclei which remained in the postincubation material (Fig. 16).

Electrone microscopy was used to analyse periblem from above the initial zone. Meristematic cells treated with dacarbazine showed some differences compared with the control (Figs. 23, 28, 33). Cytoplasm was rich in ribosomes and polyribosomes (Fig. 24). ER cisterns in parallel patterns were present in the cytoplasm (Figs. 27 and 36). Numerous mitochondria, which divided frequently, were characterized by dense matrix and varying amounts of cristae (Fig. 24). Numerous dictyosomes were also observed after dacarbazine treatment. Their cisternae contained vesicle swellings, both at the ends and in the central areas (Fig. 25).

Increase of DTIC concentration caused in visible changes of mitochondria morphology and internal structure. Most of them were noticeably swollen, whereas cristae stucked together (Fig. 26). Also number of vacuoles increased under the effect of DTIC (Figs. 29-32). Cell wall slightly thickened, and plasmalemma is frequently pulled out of the wall. Numerous vesicles with dense content were observed in aplasmatic zones, between cell wall and plasmalemma. Most probably they originated from ER or Golgi apparatus (Figs. 34 and 35).

Table 2

Effect of DTIC on respiration of meristematic cells of root tips of *Allium cepa* L.
(time of treatment—24 h)

Experimental series	Respiration, % of control (mean values)	
	DTIC, 500 ppm	DTIC, 750 ppm
1	65.32	52.59
2	58.12	53.82
3	48.55	66.11
4	47.32	55.75
5	49.34	47.25
6	55.75	53.72
7	65.79	53.53
8	50.14	47.15
9	63.57	46.28
10	51.98	33.82
Mean	55.58	51.00

The highest DTIC concentration (1000 ppm) resulted in further vacuolization of the cytoplasm. Newly formed vacuoles were optically empty (Fig. 37). ER cisternae underwent fragmentation, forming vesicles or short fragments, swollen at the end (Fig. 39). Mitochondria were visibly swollen, cristae short, and they underwent fragmentation or sticking (Fig. 38).

Changes induced by dacarbazine treatment as regards the number and structure of mitochondria suggest that DTIC may affect respiratory processes in the cell. Data presented in Table 2 show that level of cell respiration after 24 h treatment with DTIC was reduced by about 50% of the control level, both at 500 and 750 ppm concentration of DTIC. Hence, DTIC had the same effect as respiratory inhibitors.

DISCUSSION

Despite the existence of several papers on good clinical results obtained with DTIC in oncological therapy (both mono- and complex), mechanisms of the effect of this substance are still under discussion. K o k o s c h k a (1978) suggested that dacarbazine may become an anti-metabolite (purine analogue) or an alkylator, depending on the conditions of its activation. Nevertheless, mechanism of chemical reaction of dacarbazine activation seems of no importance as concerns some cytological effects. This is caused by the fact that in both cases nucleic acids are attacked by dacarbazine. Nucleic acids are either alkylated or anti-metabolite becomes incorporated into their molecule. Independently of the mechanism of DTIC effect, DNA synthesis is always inhibited. This results in disturbances of the mitotic cycle of cells, expressed by mitodepression or total blocking of the divisions. In the experimental conditions there was a visible mitostatic effect, dependent on DTIC concentration and duration of treatment. Mitostatic effect was caused, among other, by an inhibition of DNA synthesis, as illustrated by autoradiography. The other cause was a specific effect of DTIC upon the G_2 phase. After short (0.5-1 hour) postincubation in water, rapid increase of the number of divisions was observed, with partial synchronization of phases, similar as described by N o v a k et al. (1979) for 5-aminouracil treatment.

The experiments showed also that dacarbazine affects phase M in the cell life, resulting in disturbances of karyokinesis and cytokinesis. Numerous prophase, with loose or only slightly spiral pattern of the chromosomes, appeared in the material treated with DTIC. This fact suggests that process of chromatin spiralization was slowed down, as a result either of DNA alkylation by dacarbazine, or dacarbazine incorporation as purine analogue into DNA.

Fig. 17. Incorporation of H^3 -thymidine. Control, 1000 $\times$

Fig. 18. Incorporation of H^3 -thymidine. DTIC 750 ppm, incubation — 24 h, 1000 $\times$

Fig. 19. Incorporation of H^3 -thymidine. DTIC 750 ppm, incubation — 24 h, postincubation — 24 h, 1000 $\times$

Fig. 20. Incorporation of H^3 -leucine. Control, 1000 $\times$

Fig. 21. Incorporation of H^3 -leucine. DTIC 750 ppm, incubation — 24 h, 1000 $\times$

Fig. 22. Incorporation of H^3 -leucine. DTIC 750 ppm, incubation — 24 h, postincubation — 24 h, 1000 $\times$

Fig. 23. Control. 2% KMnO_4 , 12 000 $\times$

Fig. 24. Swollen mitochondria with thick matrix and varying number of cristae, numerous ribosomes. DTIC 500 ppm, incubation — 24 h, GA according to Karnovsky (1965), 20 000 X

Fig. 25. Dictyosomes with variable structure. DTIC 500 ppm, incubation — 24 h, 2% KMnO_4 , 20 000 X

Fig. 26. Disorganization of internal mitochondrial membrane. DTIC 750 ppm, incubation — 24 h, 2% KMnO_4 , 15 000 $\times$

Fig. 27. Hypertrophy of ER membranes forming parallel structures. DTIC 750 ppm, incubation — 24 h, 2% KMnO_4 , 15 000 $\times$

Fig. 28. Control. GA according to Karnovsky (1965), 2500 $\times$

Figs. 29-31. Vacuolization of the protoplast. DTIC 750 ppm, incubation — 24 h,
GA according to Karnovsky (1965), 2500 $\times$

Fig. 32. Strongly vacuolized cytoplasm, mitochondria with thick matrix, numerous ribosomes. DTIC 750 ppm, incubation — 12 h, GA according to Karnovsky (1965), 42 000 $\times$

Fig. 33. Numerous ribosomes, poliribosomes and mitochondria, Control, GA according to Karnovsky (1965), 45 000 $\times$

Figs. 34 and 35. Aplanatic area, close to the wall vesicles with thick content. DTIC 750 ppm, incubation — 24 h, GA according to Karnovsky (1965), 42 000 $\times$

Fig. 36. ER in parallel patterns, thick cell wall. DTIC 500 ppm, incubation — 24 h, 2% KMnO_4 , 15 000 $\times$

Fig. 37. Vacuolization and disorganization of the cytoplasm. DTIC 1000 ppm, incubation — 24 h, 2‰ KMnO_4 , 20 000 $\times$

Fig. 38. Mitochondria with changed cristae structure. DTIC 1000 ppm, incubation — 24 h, 2‰ KMnO_4 , 20 000 $\times$

Fig. 39. ER fragmentation. DTIC 1000 ppm, incubation — 12 h, 2‰ KMnO_4 , 20 000 $\times$

Dacarbazine had a visible effect upon mitotic apparatus of the dividing cell, depending on DTIC concentration. Types of disturbances, and especially patterns of meta- and anaphase chromosomes, allow for assuming that disorganization of the karyokinetic apparatus takes place during incubation. This suggestion is supported by comparison of pictures obtained after DTIC treatment with figures given by Östergren (1944, 1950) after treatment with colchicine, or by Tarkowska (1977) after treatment with water solution of oleander glycosides. These authors observed similar patterns of chromosomes, among others as a result of destruction of microtubules, as also of their changes at kinetochores. Parallel configuration of chromosome threads bandels, observed in light microscope, suggests destruction of the spindle, but does not allow for any further conclusions. It seems that treatment with DTIC results in only slight damage of kinetochores, reflected by their delayed division (figures similar to c-Metaphase and c-Anaphase).

DTIC treatment results also in disturbances of the cytokinesis, leading into fusion of telophase nuclei, formation of partial primary walls, and formation of binuclear cells.

These disturbances of the mitosis suggest that dacarbazine has similar effect as alkylating preparates, for which chromato- and mitoclassical effects have been described (Broda and Kocik 1972, Podbielkowska and Nowaczek 1979). Damage of the mitotic apparatus might then be considered as a result of alkylation of sulphhydryl groups of the spindle proteins.

The other fact observed in our studies was blocking of G_2 phase as one of the causes of mitodepression. On the other hand, it was not possible to establish whether the observed inhibition of DNA synthesis was caused by alkylation of nucleic acids or by incorporation of purine analogue into DNA.

Dacarbazine showed some properties as respiratory inhibitors. This was reflected not only by the results of measurements of respiration intensity, but also by characteristic changes in submicroscopic organization of the cells.

Mitochondria showed visible changes within membrane organization, especially the internal one. Significant enlargement of the perimitochondrial zone was noticeable, as well as swelling of cristae and condensation of matrix. Such structure is typical of metabolic states of ATP deficiency, occurring as a result of conformation changes in enzymatic proteins (Hackenbrock 1966).

Changes observed within Golgi apparatus also point to a deficiency of highly energetic compounds. They were similar to those described by Robinson and Ray (1977) who showed changes of structure and functioning of dictyosomes under the effect of typical respiratory inhibitors, such as KCN, NaN_3 or 2-4 DNP.

Changes within ER cisternae, consisting of initial hypertrophy and subsequent fragmentation and vacuolization, were described also as an effect of antimitotic substances, as chloralhydrate (Molè-Bajer 1969) or oleander glycosides (Tarkowska 1977), during necrosis (Wrischer 1965), and also in case of cell energetic deficiency induced with various methods (Podbielkowska and Kupidłowska 1976).

Behaviour of such plasmatic membranes as plasmalemma, characteristically detaching from the cell wall, and also accumulation of membrane or vesicle structures in aplasmatic areas, were similar to those described by Park et al. (1976) induced by toxins.

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*Aktywność mitotyczna, organizacja submikroskopowa i oddychanie
komórek merystematycznych z wierzchołków wzrostu korzeni
Allium cepa L. poddanych działaniu dakarbazyny*

Streszczenie

Badano skutki działania dekarbazyny na aktywność mitotyczną, metabolizm oddechowy oraz submikroskopową organizację komórek merystematycznych z wierzchołków wzrostu korzeni cebuli (*Allium cepa* L.). W warunkach przeprowadzonego doświadczenia DTIC wykazuje efekty mitostatyczne zależne od stężenia i czasu działania związku. Mechanizmem doprowadzającym do mitodepresji jest inhibicja syntezy DNA oraz blokada fazy G_2 . Dekarbazyna wywołuje także zaburzenia w przebiegu kario- i cytokinezy zbliżone do efektów kolchicynowych. DTIC w znacznym stopniu hamuje oddychanie komórkowe oraz powoduje charakterystyczne zmiany w submikroskopowej organizacji komórek. Cytoplazma, bogata w rybosomy i polirybosomy, stopniowo ulega wakuolizacji. Przy niższych stężeniach DTIC zauważono hipertrofię ER, a następnie jego wakuolizację i fragmentację analogicznie jak przy nekrozach lub działaniu substancji antymitotycznych i toksycznych. Diktiosomy występujące w dużych ilościach zawierały liczne pęcherzyki zarówno w częściach centralnych, jak i końcowych odcinkach cystern. Charakterystycznym przemianom ulegają mitochondria. Początkowo są one bardzo liczne i zawierają dużą ilość krist. Przy wyższych koncentracjach pęcznieją, a wewnętrzna błona ulega fragmentacji tracąc typowy kształt i układ. Opisana reorganizacja w submikroskopowej strukturze komórek jest w znacznym stopniu podobna do opisywanej po działaniu inhibitorów oddechowych.