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The Effect of Vinblastine on the Autophagic Processes in Mouse Hepatocytes

Summary

The vinblastine effect on the size of autophagy in a mouse liver cell was studied. The activity of cathepsin D and L, lysosomal esterase, N-acetyl-B-D-glucosaminidase, beta-glucuronidase acid phosphatase was determined. In an isolated lysosomal fraction, it was found that vinblastine caused an increase of protein degradation of mouse hepatocytes and this action was dependent on dose and time of exposure. It was related to an increase in lysosomal compartment.

Introduction

There is no doubut that the lysosomal system is a specific protection of the cell from stressogenic agents (Klasing 1985, Miyawaki 1988, Pfeifer 1987), it also participates in the liquidation of used organelles. In physiological conditions, there occurs, however, a process of controlled destabilization of these structures through autophagy (Glaumann 1984, Mortimore et al. 1989). Autophagy creates a specific mechanism, so-called increased or enforced degradation (Dunn 1990a, b, Gordon and Seglen 1988, Kopitz et al. 1990 Marzella and Glaumann 1987). A great number of factors including vinblastine affects the size of autophagy in the liver cell of a mouse (Glaumann and Trump 1976, Reunanen et al. 1985, Król 1993).

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In currently available literature there is no data on how vinblastine influences autphagic processes in mouse hepatocytes.

Vinblastine (VBL) is, apart from vincristine, one of the most important dimeric alkaloids of Catharanthus roseus (Kolhmünzer 1985).Vinblastine (plant alkaloid) belongs to antitubuline compounds which cause degradation of microtubules and inhibit the mitotic processes. It causes a considerable increase in the lysosomal compartment of the liver, the epithelial cells of kidney, and others (Marzella and Glaumann 1980a; Reunanen et al. 1985). As a consequence of its action intensive separation of cellular structures occurs as well as degradation in autophagic vacuoles.An attempt is made to follow the influence of vinblastine on the biochemical and morphological changes of autophagic system of the mouse hepatocytes.

Material and methods

The study was carried out on the Porton mice whose body weight was 20–25 g. Experimental animals received vinblastine intraperitoneally in 0.9% NaCl (Vinblastine, Richter, Hungary) in a concentration of 5 mg/kg of body weight. The control group received only the physiological solution. The animals were killed 4 and 24 hr after the administration of vinblastine.

The livers of control and experimental mice were homogenized at 4°C in a homogenizer with a teflon piston of the Potter's type at 200 rotations/min. Differential fractioning was carried out according to the methods of Marzella and Glaumann (Marzella and Glaumann 1980a). The protein was measured in the lysosomal fraction by a modified Lowry method (Kirschke and Wiederanders 1984), and proteolytic activity, measured as the activity of cathepsins D (EC 3.4.22.15.), and L (EC 3.4.23.5.) by Langner method (Langner et al. 1973). The activity of the lysosomal esterase (LE) (EC 3.1.1.-), N-acetyl-B-D-glucosaminidase (NAGL) (EC 3.2.1.30), beta-glucuronidase (BGRD) (EC 3.2.1.31), acid phosphatase (EC 3.1.3.2) was determined according to the Barrett method (Barrett 1972).

The activity of the investigated hydrolases was expressed in nanomoles per milligram of protein per hour (nmol/mg/hr). Immediately after decapitation, a slice of the liver of animals was taken for microscope studies. Fixation and osmication were performed according to the method of Marzella and Glaumann (Marzella and Glaumann 1980a).

Results

The activity of the investigated lysosomal enzymes in the livers of control mice was assumed to be 100%. The ultrastructure of the liver cells of those animals corresponds with that of the unexcited liver cell.

Tab. 1. The influence of vinblastine /5mg/k.b.wt/ on the activity of some lysosomal hydrolases in the mouse liver /nmol/mg protein/hr/ given as per cent changes in relation to the control group taken as 100%

Enzyme	n	Control group		After Vinblastin 4hr		After Vinblastin 24hr	
		x ± SE	%	x ± SE	%	x ± SE	%
Proteolytic activity	20	0.876 ± 0.166	100	1.218 ± 0.097**	139	1.462 ± 0.168***	160
LE	20	3.760 ± 0.714	100	3.497 ± 0.314	93	5.978 ± 0.478***	159
KF	20	2.420 ± 0.435	100	2.638 ± 0.520	109	2.541 ± 0.460	105
NAGL	20	0.480 ± 0.091	100	0.470 ± 0.042	98	0.461 ± 0.037	96
BGRD	20	2.710 ± 0.379	100	3.144 ± 0.251*	116	2.312 ± 0.169*	89

*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

As can be seen from Tab. 1, the vinblastine effect on the proteolytic activity of the investigated enzymes had two-stages, and it manifested itself in the long time intervals. Already after 4 hr from the administration of vinblastine, a statistically confirmed increase of catheptic activity was observed in comparison with the control group, an average of 39%, and after 24 hr 60%.

The activity of lysosomal esterase was statistically significantly increased only after 24hr, at an average of 59% whereas no significant changes were found after 4 hr although a marked induction of autophage vacuoles (Figs 1 and 2) occurred at that time.

The activity of acid phosphatase and *N* -acetylo-beta-glucosaminidase in the period of 4hr after vinblastine administration did not change markedly. However, a slight increase of the activity of beta-glucuronidase has been observed. Administration of vinblastine caused, however, an increase in autophagic processes. The newly-formed autophagic vacuoles were limited by different endoplasmatic membranes (Fig. 3).

Autophagosomes and typical autophagic vacuoles are visible there. Endoplasmic reticulum and mitochondria were prevailing content of these autophagic vacuoles together with other recognizable components of cytosol.

After 24 hr vinblastine load, we observed the accumulation of density endogenic lipoproteins in the hepatocytes of the investigated mice. The ultrastructure of the liver cells presented in the electrograms indicates a state of stimulation of the biosynthetic processes which are manifested, amongst others, by a proliferation of the rough endoplasmic reticulum (Fig. 4).

Discussion

The lysosomal compartment is the main place of intercellular catabolic processes (Hirsimake et al. 1976, Mortimore et al. 1989) which form a specific compensation system of a cell subjected to unfavourable conditions of existence (Gordon et al. 1989, Seglen 1987, Seglen et al. 1992). Cellular compensatory mechanisms at the level of lysosomal space are first of all processes of autophagocytosis which can run with a considerable increase of proteolysis and lipolysis (Pfeifer 1981, 1987). In mammals the crucial role is played by the lysosomal system of the cells of liver, muscules and partly kidney (Mortimore et al. 1977, 1989).

It has been mentioned that vinblastine increases the processes of autophagy in a eucariotic cell and thereby it contributes to the rise of cell proteolysis and lipolysis. On the basis of results obtained, one can see a distinct influence of vinblastine on the proteolytic activity of liver cells in mice (Table 1). Using the method of Langner only the activity of cathepsin D and L is estimated, since the other proteases are inactive under these conditions. The presence of both cathepsin in liver cells was previously estimated (Barett and Kirschke 1981). The vinblastine influence on the rise of proteolytic-activity had two phases and reached averagely 39% after 4 hr, and 60% after 24 hr. Thus proteolytic activity underwent a considerable stimulation within 24 hr after vinblastine administration. Some authors (Glaumann 1984, Marzella and Glaumann 1980a, b 1987), did not find, however, marked increase of proteolytic activity within 4 hr after vinblastine administration. Perphaps it is the result of use of another method of proteolysis estimation by those authors. Four hour vinblastine effect also brought a considerable increase of autophagy processes, which are conformable with the reports of Hirsimäke et al. (1976), Kopitz et al. (1990), and Pfeifer (1981).

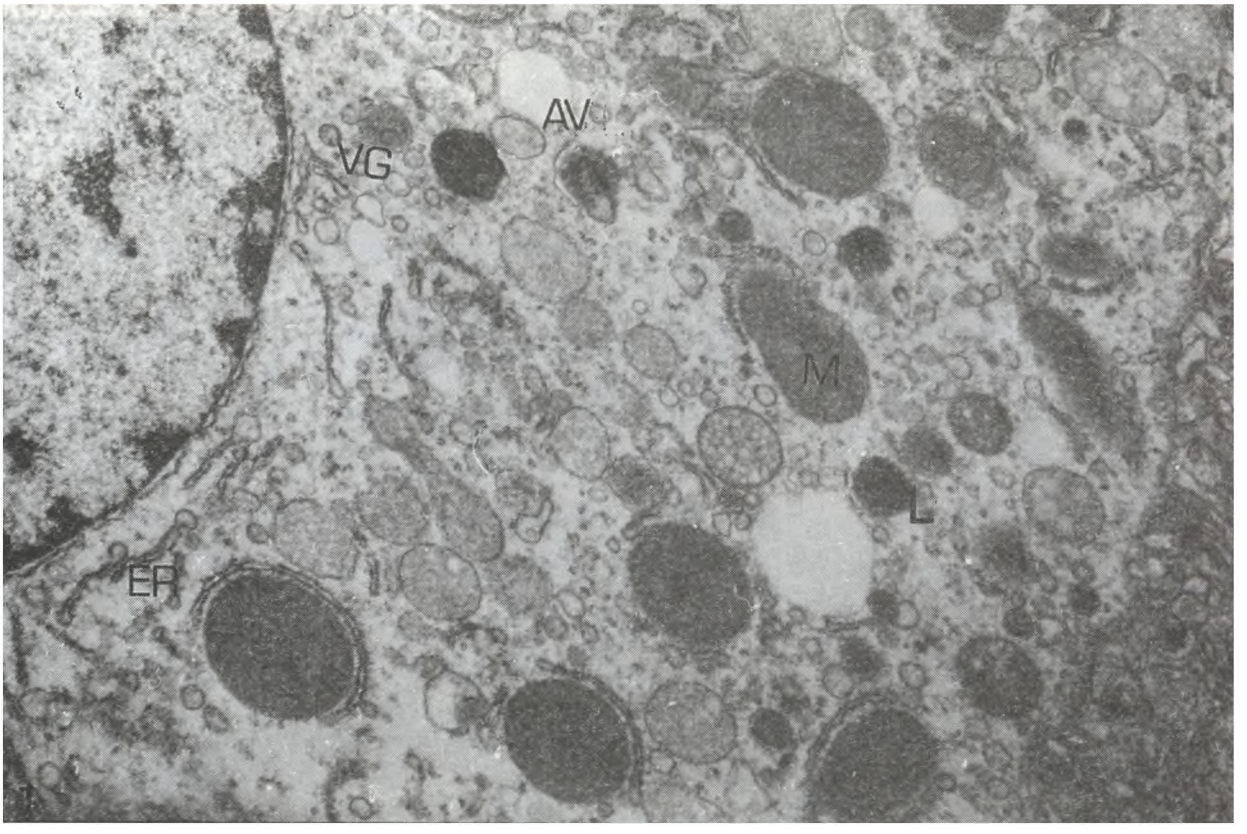

Fig.1. Electron micrographs of hepatocytes 4hr after vinblastine treatment.
(L): lysosome; (M.): mitochondria ;(AV): autophagic vacuole: (VG) Golgi complexes with VLDL: (ER): enoplasmatic reticulum, x 20.000

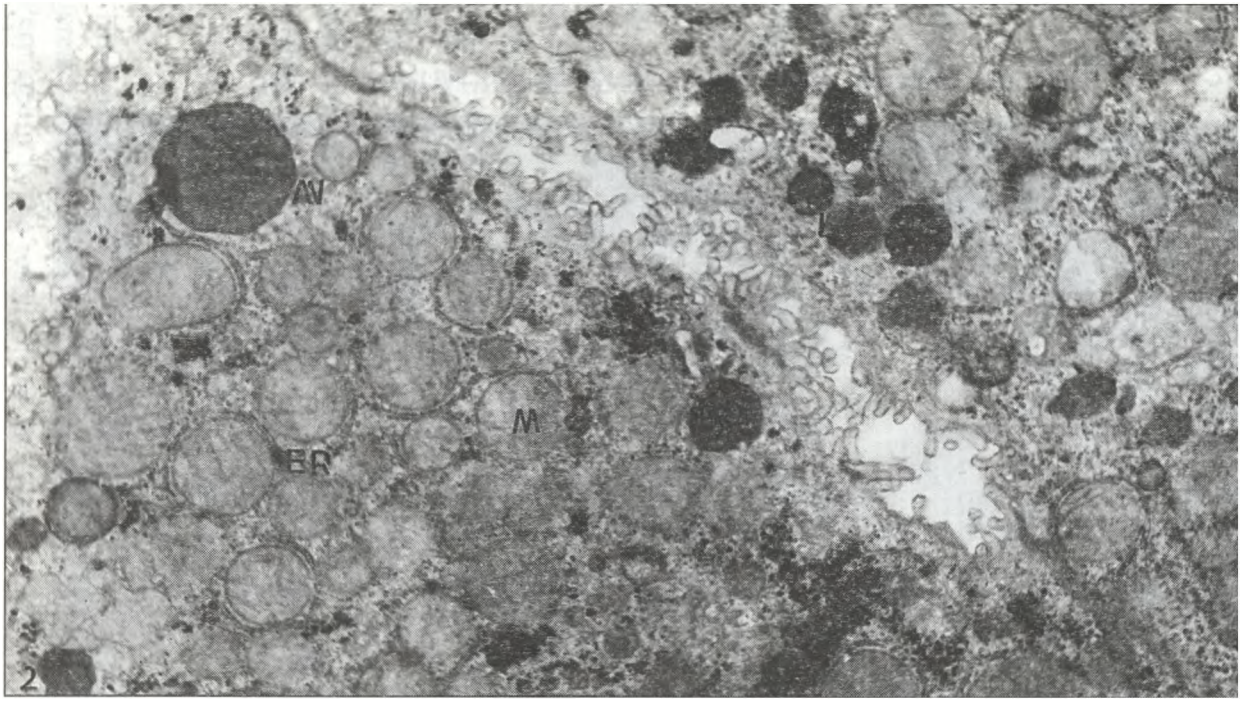

Fig.2. Electron micrographs of hepatocytes 4hr after vinblastine treatment . (L): lysosome;(M.): mitochondria; (AV): autophagic vacuole; (ER): endoplasmatic reticulum, x 18.000

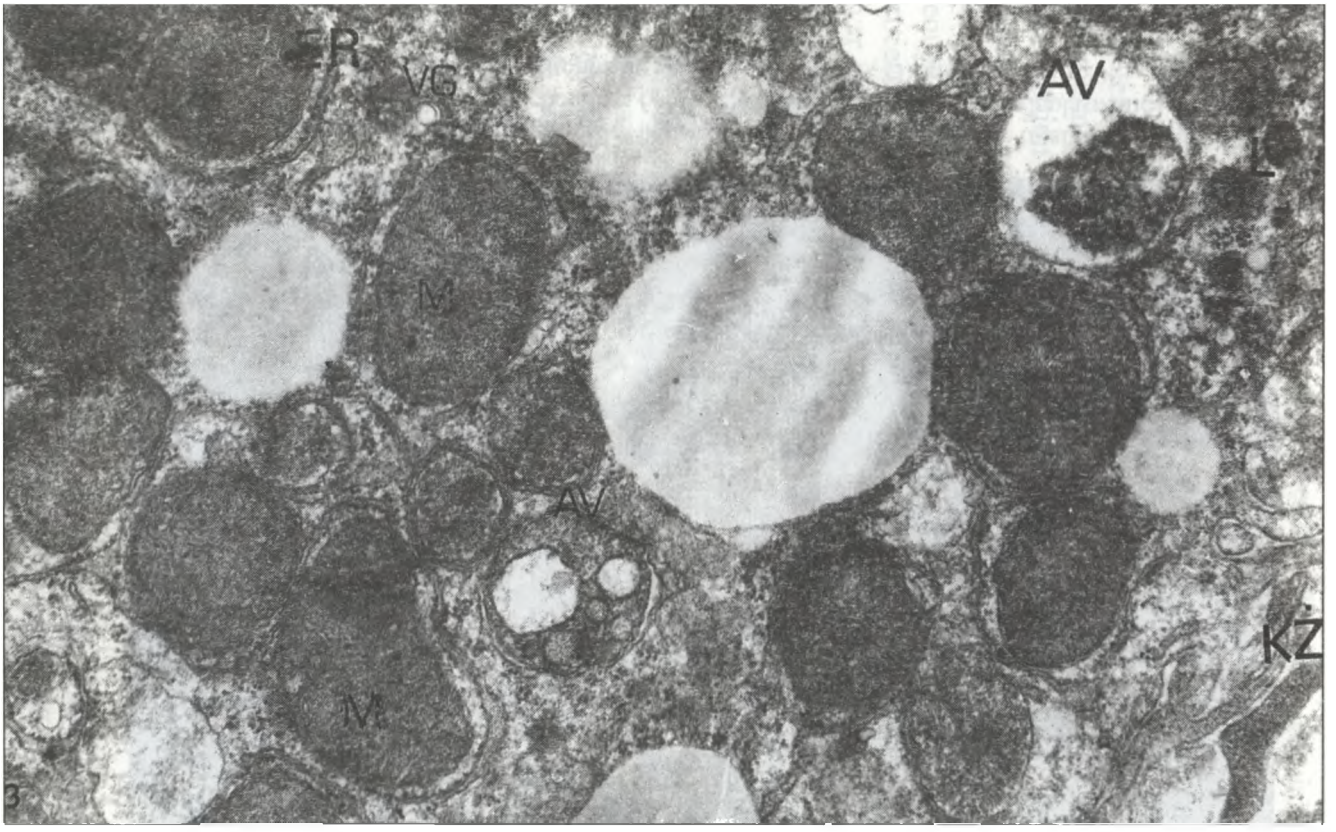

Fig.3. Electron micrographs of hepatocytes 24hr after vinblastine treatment.
(L): lysosome ;(M): mitochondria ;(AV): Autophagic vacuole ;(ER): endoplasmatic reticulum, (VG): autophagic vacuole with VLDL; (R) ; ribosome; x 25.000

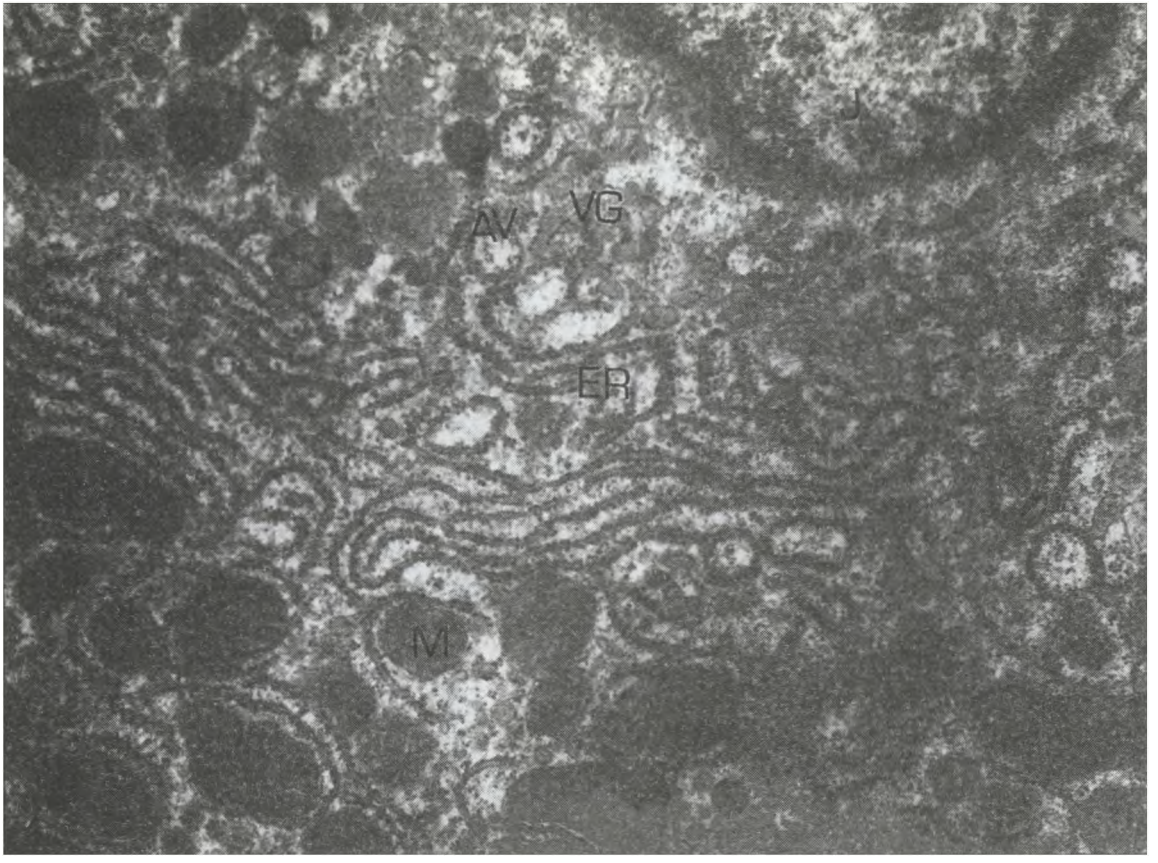

Fig.4. Electron micrographs of hepatocytes 24 hr after vinblastine treatment. (L): lysosome; (M): mitochondria; (AV): autophagic vacuole;(ER): endoplasmatic reticulum, (VG): autophagic vacuole with VLDL; (J): nucleus; x 13.200

It fallows unequivocally from our investigation that a considerable simulation of proteolytic activity was observed after 24 hr of vinblastine effect. Specific activity in hepatocytes increased from the value 0.876 nmoles/mg of protein/hr to 1.462 nmoles/mg of protein/hr. This increase can accounted for amongst others, by an increase of the biosynthesis of proteolytic enzymes although this increase is prolonged in time in comparison to autophagy vacuoles induction. These results confirm the suppositions of Marzella and Glaumann (1980) that only the beginning of lipolyses occurs in the lysosomal fraction and the final degradation of lipids occurs in the cytoplasmic space. A number of authors (Marzella and Glaumann 1980a, b, Mortimore and Schworer 1977, Pfeifer 1981, Reunanen et al. 1985) found that in consequence of vinblastine administration there occurs an increase in autophagy processes linked with a degradation of subcellular structures in the cells of liver and the epithelium of kidney tubules. The rate of these degradation processes is dependent on the activity of proteolytic and lipolytic enzymes.

It has been shown that lysosomes are capable of degrading all subcellular structures, and moreover, the level of degradation induced by vinblastine is dependent on dose and time of reaction. These data are conformable with the conclusions of Marzella and Glaumann (1980a), Pfeifer (1981, 1987) and Reunanen et al. (1985).

They found that it was possible to measure the proteolysis increase 30 min after the administration of a large dose of vinblastine (5mg/100g of the body weight). After 4 hr these values were 2 or 3 times greater than control values. It was shown on the model of liver that changes in the rate of protein degradation occur in parallel with changes in the size and appearance of the lysosomal compartment (Neely et al. 1977).

It may be suggested on the basis of the obtained results that vinblastine caused an increase in the general degradation ability of the mouse hepatocytes, and its action depended on the dose and time of duration.

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Wpływ vinblastyny na procesy autofagowe hepatocytów wątroby myszy

Streszczenie

Vinblastyna należy do najważniejszych alkaloidów dimerycznych *Catharanthus roseus*. Posiada właściwości cytostatyczne i jest jednym z najważniejszych związków wykorzystywanych w leczeniu nowotworów. Dotychczas wiadomo, że vinblastyna hamuje mitozę w stadium metafazy dając efekt C-mitotyczny. Jest on

następstwem interakcji vinblastyny z białkami aparatu wrzeciona kariokinetycznego i mikrotubul, jednak mechanizm jej działania nie jest do końca poznany.

W literaturze jest również niewiele danych dotyczących wpływu vinblastyny na procesy autofagowe.

W niniejszej pracy prześledzono wpływ vinblastyny na zmiany biochemiczne i ultrastrukturalne w wątrobie myszy jednego gatunku po 4 i 24 godzinach działania tego alkaloidu. Zmiany biochemiczne przeanalizowano badając aktywność wybranych hydrolaz lizosomalnych: katepsyny D i L (E C 3.4.23.5, E C 3.4.22.15), N-acetyl-B-D-glukozaminidazy (E C 3.2.1.30), betaglukuronidazy (E C 3.2.1.31), esterazy lizosomalnej (E C 3.1.1.-) oraz kwaśnej fosfatazy (E C 3.1.3.2). Jednocześnie od tych samych zwierząt pobierano wycinki do badań ultrastrukturalnych.

Obserwowane istotne zmiany morfologiczne pozostawały w ścisłej korelacji z uzyskanymi wynikami biochemicznymi. Stwierdzono, że wzrost procesów autofagowych miał różnorodny zakres i uzależniony był od czasu działania użytego alkaloidu.