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Cytotoxic effects of cantharidin on the growth of normal and carcinoma cells

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Abstract

Cantharidin is isolated from *Mylabris phalerata* Pallas and is a potent inhibitor of hepatocellular carcinoma cells (Hep 3B cells). In the present study, the IC_{50} values of cantharidin on Hep 3B cells and normal Chang liver cells were found to be 2.2 and 30.2 μM for 36 h, respectively. Furthermore, cantharidin-treated Hep 3B cells induced cell death within 1 h ($IC_{50} = 52.8$ μM), suggesting that cantharidin is an acute cytotoxic agent. We found that although cantharidin could induce cell death, it could not directly inhibit the activity of nucleic acid biosynthesis by the cellular incorporation of ^{3}H-thymidine, ^{3}H-uridine or ^{3}H-leucine. Cantharidin-treated Hep 3B cells showed no evidence of major alterations in the cell cycle distribution within 1 h. However, examination of cells after treatment for 36 h showed that cantharidin regulated the cell cycle at the G_2/M phase. Moreover, the treated Hep 3B cells had a rounded and shrunken appearance. The microvilli of treated Hep 3B cells were reduced in number and replaced by numerous blebs. Other ultrastructural changes following cantharidin treatment included the presence of lipid droplets, swelling of the mitochondria and accumulation of glycogen particles. The findings of damaged mitochondria in the cantharidin treated Hep 3B cells in this study suggest that cantharidin can induce acute and lethal toxic effects on Hep 3B cells by inhibiting the mitochondria energy system. In conclusion, this study had demonstrated that cantharidin could inhibit progression of all phases of the Hep 3B cell cycle.

Keywords: Cantharidin; Hepatocellular carcinoma; Cytotoxicity; Nucleic acid biosynthesis; Ultrastructure

1. Introduction

Mylabris is the dried body of the Chinese blister beetle (*Mylabris phalerata* Pallas). Since ancient times, the crude drug of Mylabris has been noted for its highly irritant action and, if swallowed, has been reported to produce congestion

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of the urethral mucosa, which may result in priapism in men and pelvic congestion in women (Polettini et al., 1992). For these reasons, Mylabris has been employed in the treatment of impotence and for many years was considered to be an aphrodisiac (Hundt et al., 1990). Other traditional uses of Mylabris include treatment of poor local blood circulation, dropsy, pleurisy, pericarditis and amenorrhea in Europe and China (Wang, 1989; Hundt et al., 1990). The active agent of Mylabris is cantharidin (hexahydro-3aα, 7aα-dimethyl-4β, 7β-epoxyisobenzofuran-1, 3-dione, Fig. 1). In recent studies, cantharidin was found to possess antitumor activities and to increase the number of leucocytes (Walter and Cole, 1967; Xu, 1981). For example, cantharidin inhibited the growth of some tumor cells including HeLa cells, murine ascites hepatoma, and reticulocell sarcoma (Xu, 1982; Huang et al., 1997). Clinical trials have indicated that cantharidin had antitumor effect on patients with primary hepatoma, but this application was limited by its severe toxicity to mucous membranes, mainly in the gastrointestinal tract, the ureter and the kidney (Oaks et al., 1960; Graziano et al., 1987; Wang, 1989). The clinical dosage for the treatment of hepatoma was 0.5 mg/day and the LD_{50} was 30 mg/kg in humans (Wang, 1989). However, the anticancer effects of cantharidin on hepatoma cells remain unknown. Although cantharidin has been shown to be a protein phosphatase inhibitor (Li et al., 1993; Eldridge and Casida, 1995; Liu et al., 1995), the mechanism by which cantharidin promotes cell death is unknown.

In the present phytochemical biological study, cantharidin was isolated from *M. phalerata* Pallas and its cytotoxic effects on human carcinoma cell lines (KB, Hep 3B, DU-145, HeLa, AGS) and normal cell lines (WISH, Chang Liver Cells) were examined. The purpose of the present study was to explore how cantharidin induces cell death in Hep 3B cells. The toxic effects of cantharidin were assessed by examining alterations of DNA, RNA and protein biosynthesis, as well as cell cycle distribution of Hep 3B cells. Then the ultrastructural alterations of cantharidin-treated Hep 3B cells were also observed by transmission electron microscopy.

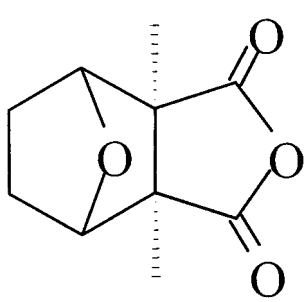

Fig. 1. The structure of cantharidin.

2. Materials and methods

2.1. Cell culture

The human hepatocellular carcinoma cell line Hep 3B, human oral epidermoid carcinoma KB, human prostate carcinoma DU-145, human cervical carcinoma HeLa, normal human amnion WISH and human normal Chang liver cell lines were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA) and were grown in plastic tissue culture plates using Dulbecco's modified Eagle medium (GIBCO BRL, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (HyClone, Logan, UT, USA), 100 U/ml penicillin–streptomycin (GIBCO BRL) in a humidified atmosphere containing 5% CO_2 in air at 37°C.

2.2. Isolation of cantharidin

The dried insect (6 kg) of *M. phalerata* Pallas purchased from the traditional Chinese medicinal market in Taipei. It was milled and refluxed with 50% aqueous ethanol (40 l) twice. The hot extract was filtered and concentrated with rotary evaporator to remove ethanol. The aqueous solution was then extracted with ethyl acetate. After concentrating and standing for over night, a white precipitate was obtained. Furthermore, it was adapted silica gel column chromatography with $CHCl_3$–MeOH gradient and purified with ethyl acetate to give a colorless needle crystal, m.p. 218°C sublimes at 110°C (12.25 g). The instru-

ment analysis data were shown as the following, EI–MS m/z, 96, 128, 197 $[M+H]^+$. IR (KBr) cm^{-1}, 3000 (C–H), 1780–1850 (C=O), 1240 (C–O). ^{1}H–NMR (acetone-d_6, 300 MHz) δ, 1.25 (6H, s, CH_3), 1.7–2 (2H, m, H-5, H-6), 4.65 (2H, t, $J=4.9$ Hz, H-4, H-7). ^{13}C–NMR (acetone-d_6, 75 MHz) δ, 12.45 (CH_3), 23.69 (C-5, C-6), 56.20 (C-3a, C-7a), 85.95 (C-4, C-7), 177.28 (C-1, C-3). On the basis of these data and comparing with the literatures (Stork et al., 1953; Walter and Cole, 1967; Huang et al., 1997) was proposed, and this was identified as cantharidin by high-performance liquid chromatograph (HPLC), GC–MS profile and nuclear magnetic resonance spectra with a authentic cantharidin (Sigma, St. Louis, MO, USA) and shown the purity over 99%.

2.3. Drug preparation

Cantharidin and adriamycin (Sigma, St. Louis, MO, USA) were initially dissolved in dimethylsulfoxide (DMSO, Sigma) at a concentration of 4 mg/ml and then stored at 4°C until use.

2.4. Cytotoxicity assay

Serial dilutions of tested samples in culture medium were prepared in 96-well microtiter plates. Drug solution at the appropriate concentration was added to cell cultures (3×10^4 cells per ml) for 1, 24, 48 and 72 h without renewal of the medium. Cells were counted by tetrazolium assay (MTT kit, Boehringer Marmheim Biochemica, Germany). Finally, the products were evaluated using an ELISA microplate reader (Dynex technologies, Guernsey, Channel Islands, UK) at 600 nm. The concentrations of drugs giving 50% growth inhibition (IC_{50}) were determined from three separate experiments (Rubinstein et al., 1990).

2.5. Measurement of DNA, RNA, and protein synthesis

DNA, RNA, and protein synthesis were measured by the cellular incorporation of ^{3}H-thymidine (NET-027, Boston, MA, USA), ^{3}H-uridine (NET-174) and ^{3}H-leucine (NET-460), respectively. Isolated cells (6×10^3 cells per well) were seeded into microtiter plates overnight and then incubated with medium containing a series of concentrations of drugs. After 1 h, the supernatant was discarded and all the samples were washed once with PBS to which 2 μCi/ml radioactive precursors were added in culture. All cells were tested for the incorporation of the radioactive precursors over an identical pulse period. The incorporation of labeled precursors was stopped by adding 10% trichloroacetic acid (Sigma) and refrigerating the plates at 4°C. After 20 min, the plates were washed with ethanol and dried. The residues were dissolved in 1% sodium dodecyl sulfate (Merck, USA) in 0.3 N NaOH at 60°C for 30 min. The solutions were then placed in a 2 ml Biofluor cocktail (Ecoscint H, National Diagnostics) and counted in a liquid scintillation counter (Beckman, LS-6500). All experiments were performed in triplicate. The concentration of drug required to inhibit 50% of the uptake of ^{3}H-thymidine in the cultured cell population (ID_{50}) was calculated as previously described (Woynarowski and Konopa, 1980; Freshney, 1993).

2.6. Flow cytometry analysis

After treatments, cells were trypsinized, centrifuged, resuspended in PBS, and then gently vortexed while 95% ethanol was slowly added to a final concentration of 80%. The fixed cells were washed with PBS, and then treated with 0.25 ml of 0.5% Triton X-100 solution containing 1 mg/ml RNase A at 37°C for 30 min. Finally, 0.25 ml of 50 μM propidium iodide was added to the sample for 30 min in the dark. Samples were run through a FACScan to count the number of cells (Becton Dickinson). Results are presented as the number of cells versus the amount of DNA as indicated by the intensity of fluorescence (Darzynkiewicz et al., 1984).

2.7. Transmission electron microscopy

The hepatoma carcinoma cell line (Hep 3B) was plated in 35 mm dishes and allowed to incubate overnight. Aliquots of cantharidin (50 μM) were added into the culture dishes for 10, 30 min or 1

Table 1
IC_{50} values of cantharidin on the tumor and normal cell lines after 36 h treatment[a]

Drug	IC_{50} (μM)[b]							
	Tumor cell line					Normal cell line		
	KB	DU-145	Hep 3B	AGS	HeLa	Chang liver	WISH	SI[c]
Cantharidin	10.1	19.8	2.2	3.3	9.5	30.2	13.4	13.5
Adriamycin	1.9	<0.5	1.3	0.8	0.2	0.3	<0.5	5

[a] Data represent the mean value of three separated experiments.
[b] Drug concentration required to reduce the absorbance to 50% of that of control cultures after 36 h in culture.
[c] IC_{50} for Hep 3B cell line/IC_{50} for Chang liver cell line.

Table 2
The IC_{50} values after different intervals of drug treatment in Hep 3B cells[a]

Drug	IC_{50} (μM)				
	Drug treatment duration of Hep 3B cell line				$IC_{50-24\ h}/IC_{50-72\ h}$
	1 h	24 h	48 h	72 h	
Cantharidin	52.8	4.5	1.6	1	4.5
Adriamycin	>300	27.1	1.1	0.9	29.7

[a] Data represent the mean values of six separated experiments.

h. At the end of incubation, the cell samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in 2% buffered osmium tetroxide for 2 h and dehydrated in ethanol. Specimens used for transmission electron microscopy were embedded in epoxy. Thin sections were cut on an ultramicrotome (Reichert Ultracut E, Leica) and double stained with uranyl acetate and lead citrate. Electron micrographs were taken on a Hitachi, H-600 electron microscope operating at 75 kV.

3. Results

3.1. Cytotoxic effect of cantharidin

When the five human cancer cell lines and normal cell lines were treated with cantharidin in doses ranging from 0.5 to 25 μM for 36 h, cytotoxic activity was observed in a dose dependent manner in all cases. Table 1 summarizes the IC_{50} values for the five cancer cell lines and normal cell lines (WISH and Chang liver cell). The hepatocellular carcinoma cell line, Hep 3B, proved to be the most sensitive cell line, with an IC_{50} value of 2.2 μM, 1/10 of that (19.8 μM) found for the prostate carcinoma cell line, DU-145 (Table 1). The selective index (SI) of Hep 3B and Chang liver cells were obtained using the IC_{50} values ratio shown in Table 1. The SI of cantharidin for Chang liver cells was 13 times higher than that for Hep 3B cells. Moreover, compared with adriamycin, cantharidin showed lesser cytotoxicity in normal cell lines. The cytotoxicity of cantharidin and adriamycin at different times was studied using doses ranging from 0.1 to 300 μM. The results showed that cells under continuous exposure to adriamycin for 72 h (IC_{50} 0.9 μM) exhibited more marked effects than those with only 24 h exposure (IC_{50} 27.1 μM, $IC_{50-24\ h}/IC_{50-72\ h}$ = 29.7), but that cells continuously exposed to can-

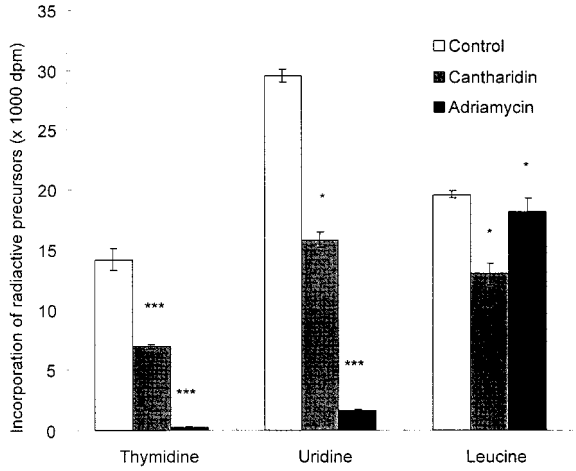

Fig. 2. The incorporation of ^{3}H-thymidine ^{3}H-uridine and ^{3}H-leucine into Hep 3B cells treated with either cantharidin (50 μM) or adriamycin (17 μM) for 1 h. Each point represents the mean of triplicate samples (mean ± S.D.). *, $P < 0.05$,**, $P < 0.005$,***, $P < 0.0005$ vs. control value.

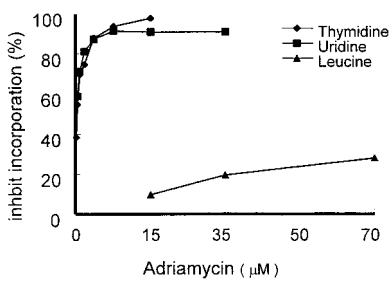

Fig. 3. Inhibition curves for the incorporation of labeled precursors into Hep 3B cells by pretreatment with a series of concentrations of adriamycin for 1 h. Each point represents the mean of triplicate samples.

Table 3
The IC_{50} and ID_{50} values of Hep 3B cell line after 1 h incubation with cantharidin or adriamycin[a]

Drug	Hep 3B cell line	
	IC_{50} (μM)	ID_{50} (μM)[b]
Cantharidin	52.8	101.5
Adriamycin	>300	0.5

[a] Data represent the mean value of three separated experiments.

[b] Drug concentration required to inhibit the uptake of ^{3}H-thymidine by 50% of cultured cell population.

tharidin did not display further cytotoxic changes (Table 2). After 24 and 72 h exposure to cantharidin, the IC_{50} of cantharidin was 4.5 and 1 μM, respectively. No discriminative changes could be detected after continuous exposure of cells to cantharidin ($IC_{50-24\ h}/IC_{50-72\ h} = 4.5$). Moreover, after 1 h exposure, the IC_{50} of cantharidin in Hep 3B cells was approximately 52.8 μM. Comparing the effects of these two agents showed that cantharidin was a more acutely lethal agent than adriamycin.

3.2. Effects of cantharidin on DNA, RNA, and protein synthesis

Hep 3B cells were treated separately with cantharidin or adriamycin. Fig. 2 shows the incorporation of ^{3}H-thymidine ^{3}H-uridine and ^{3}H-leucine into Hep 3B cells treated with either adriamycin or cantharidin. The inhibitory effect of 17 μM adriamycin on the cellular incorporation nucleic acid precursors was greater than that of cantharidin at 50 μM (Fig. 2) and was also concentration-dependent (Fig. 3). Each uptake of ^{3}H-thymidine ($P < 0.05$), ^{3}H-uridine ($P < 0.0005$) and ^{3}H-leucine ($P < 0.05$) into the Hep 3B cells was significantly inhibited by cantharidin at 50 μM (Fig. 2). Cantharidin did not affect the uptake after 1 h exposure at concentrations lower than 50 μM. Moreover, adriamycin showed a relatively quick and effectual inhibition of ^{3}H-thymidine and ^{3}H-uridine uptake in contrast to its relatively steady repression of ^{3}H-leucine incorporation (Fig. 3). The inhibition of incorporation of ^{3}H-thymidine into Hep 3B cells by cantharidin was comparable with that by adriamycin (Table 3). The ID_{50} of adriamycin was lower than the IC_{50} in adriamycin-treated Hep 3B cells, indicating that adriamycin inhibited ^{3}H-thymidine uptake ($ID_{50} = 0.5$ μM) rather than blocking cell growth ($IC_{50} > 300$ μM). Thus, cantharidin was not as effective as adriamycin in S phase inhibition and had a general cytotoxic effect.

3.3. Effect of cantharidin on cell-cycle progression

Hep 3B cells were treated with a range of

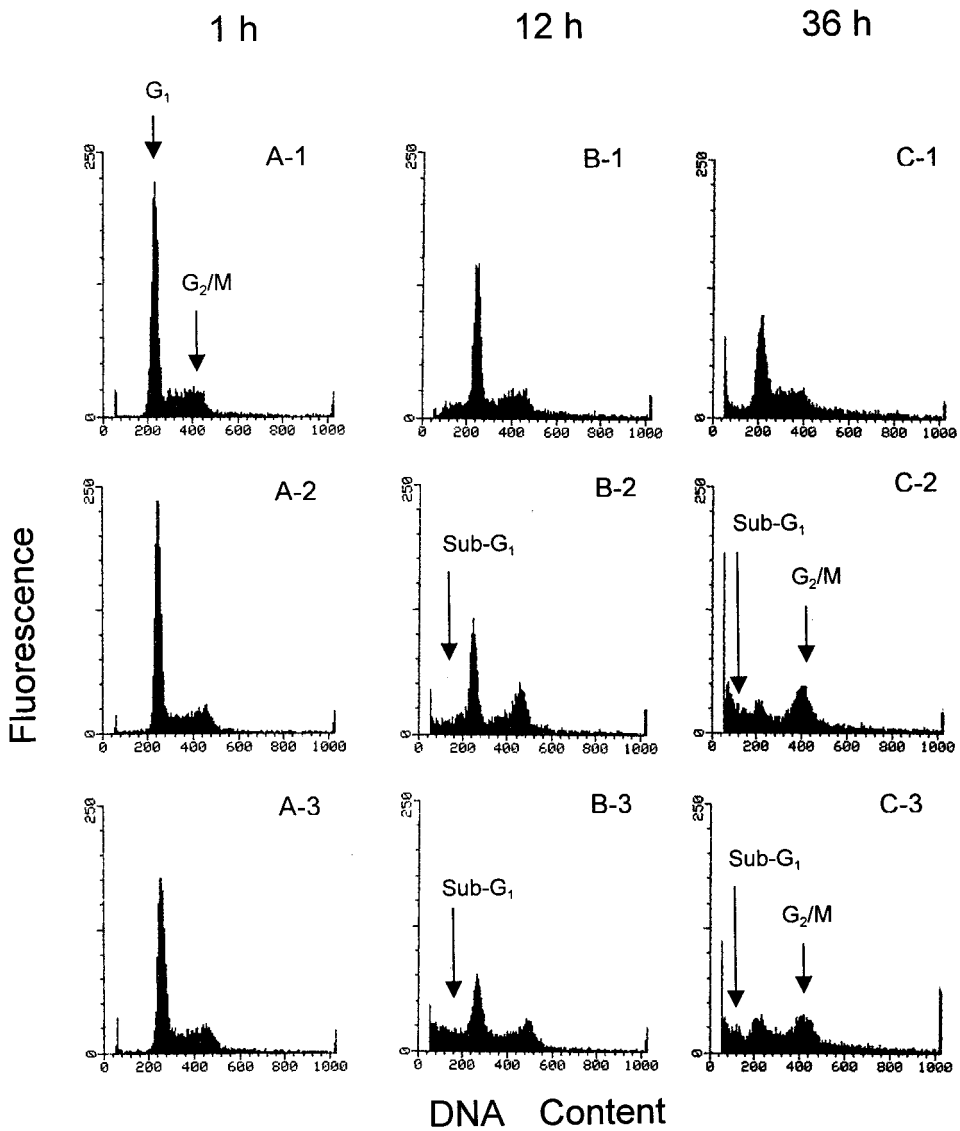

Fig. 4. Effect of cantharidin on cell cycle distribution of Hep 3B cells determined by flow cytometry. Representative DNA histograms of Hep 3B cells untreated (A-1) or exposed to 50 μM cantharidin for 1 h, attached-cells (A-2) and floating-cells (A-3) and cell untreated (B-1) or exposed to 2.5 μM cantharidin for 12 h, attached-cells (B-2) and floating-cells (B-3), and cell untreated (C-1) or exposed to 0.3 μM (C-2), 0.6 μM (C-3) cantharidin for 36 h, respectively.

cantharidin concentrations and the cell cycle distribution was examined after 1, 12 and 36 h of drug treatment. We collected floating-cells and attached-cells after cantharidin treatment of Hep 3B cells at 50 μM for 1 h. No major alterations in the cell cycle distributions were observed (Fig. 4A). When cells were treated with 2.5 μM catharidin for 12 h, a three-fold accumulation of cells in the G_2/M phase was seen in floating and viable cells (Fig. 4B). After treatment with 0.3 or 0.6 μM catharidin for 36 h, both attached and floating Hep 3B cells were collected for flow cytometry analysis and the appearance of G_2/M arrest and Sub-G_1 peaks were detected (Fig. 4C). The above results suggest that cantharidin regulated the cell cycle during the G_2/M phase and

induced cell death in treatments with low concentrations for long times. However, while cantharidin treatment induced acute cell death, the absence of a major accumulation of cells in any specific phase of the cell cycle indicated that the predominant effect of the drug was to block progression in all phases of the cell cycle.

3.4. Ultrastructural of cantharidin treated Hep 3B cells

Hep 3B cells cultured in the control medium showed many cell aggregates in which cells were closely associated with each other the microvilli. These cells displayed numerous smooth endoplasmic reticulum, Golgi apparatus and mitochondria (Fig. 5A). Hep 3B cells after 10 min incubation with 50 μM cantharidin showed increased lipid droplets but their other organelles did not exhibit emphatic change (Fig. 5B). After 30 min incubation with 50 μM cantharidin, Hep 3B cells showed fewer microvilli and induced blebs and contained some swollen mitochondria and increased glycogen particles that were concentrated in the vicinity of the Golgi apparatus or accumulated in the periphery of the cell (Fig. 5C). Severe changes of Hep 3B cells were seen after treatment with cantharidin for 1 h including necrotic organelles in the form of deformed lipid droplets, swelling of periunclear cisternae and endoplasmic reticulum, and the presence of mitochondria without intact cristae (Fig. 5D).

4. Discussion

Adriamycin is generally thought to act by intercalating into double stranded DNA (Cummings et al., 1993) and is commonly, used in clinical chemotherapeutics. In this study, adriamycin was as a positive control agent in experiments with cantharidin. The results of this study show cantharidin had lower cytotoxicity than adriamycin on normal cell lines (Table 1). The results indicate that cantharidin specifically inhibited the growth of Hep 3B cells.

The cytotoxic effects of cantharidin on Hep 3B cells were evident at 1 h of continuous exposure (Table 2), with an IC_{50} of 52.8 μM. This result indicates that cantharidin can cause acute injury to cells and that this may be due to a better diffusion of cantharidin through the cell membranes, due to its nonpolar nature and low molecular size. In contrast, the IC_{50} value of adriamycin under the same conditions was greater than 300 μM, suggesting that adriamycin did not immediately inhibit cell growth of the cell cycle.

Most anticancer drugs act by inhibition the DNA synthesis. It has been previously reported that cantharidin interferes with the metabolism of protein and nucleic acid molecules in cancer cells (Wang, 1989). The results of this study indicate a concentration-dependent inhibition of DNA and RNA biosynthesis in cells treated with adriamycin as reflected by its inhibition of ^{3}H-thymidine and ^{3}H-uridine uptake (Fig. 3). This supports the specific effects of adriamycin on the synthesis of nucleic acids. In the present study, the cytotoxic effect of cantharidin ($IC_{50} = 52.8$ μM) was seven times that of adriamycin ($IC_{50} > 300$ μM) after 1 h treatment, indicating that cantharidin is a more acutely cytotoxic agent than adriamycin. Moreover, the ID_{50} value of cantharidin in Hep 3B cells (101.5 μM) was higher than the IC_{50} (52.8 μM). These findings suggest that cantharidin does not exert its cytotoxic effects by inhibiting nucleic acid synthesis, but rather by impairment of cell function which leads to subsequent damage of DNA biosynthesis.

In this study, cantharidin demonstrated components of cytotoxic activity and caused a general block of progression of all phases of the cell cycle. This might reflect an effect on the energy availability within tumor cells, which would be detrimental to many processes of the cell cycle (Martin et al., 1994). We noted that canthardin also induced a relatively minor (three-fold) accumulation of cells in the G_2/M phase. This effect occurred at 2.5 μM exposure for 12 h and may have reflected an effect of the drug. Moreover, cantharidin has been reported to induce aberrant centrosome replication, the appearance of multiple aberrant mitotic spindles, and G_2/M phase growth arrest in a CHO cell line (Cheng et al.,

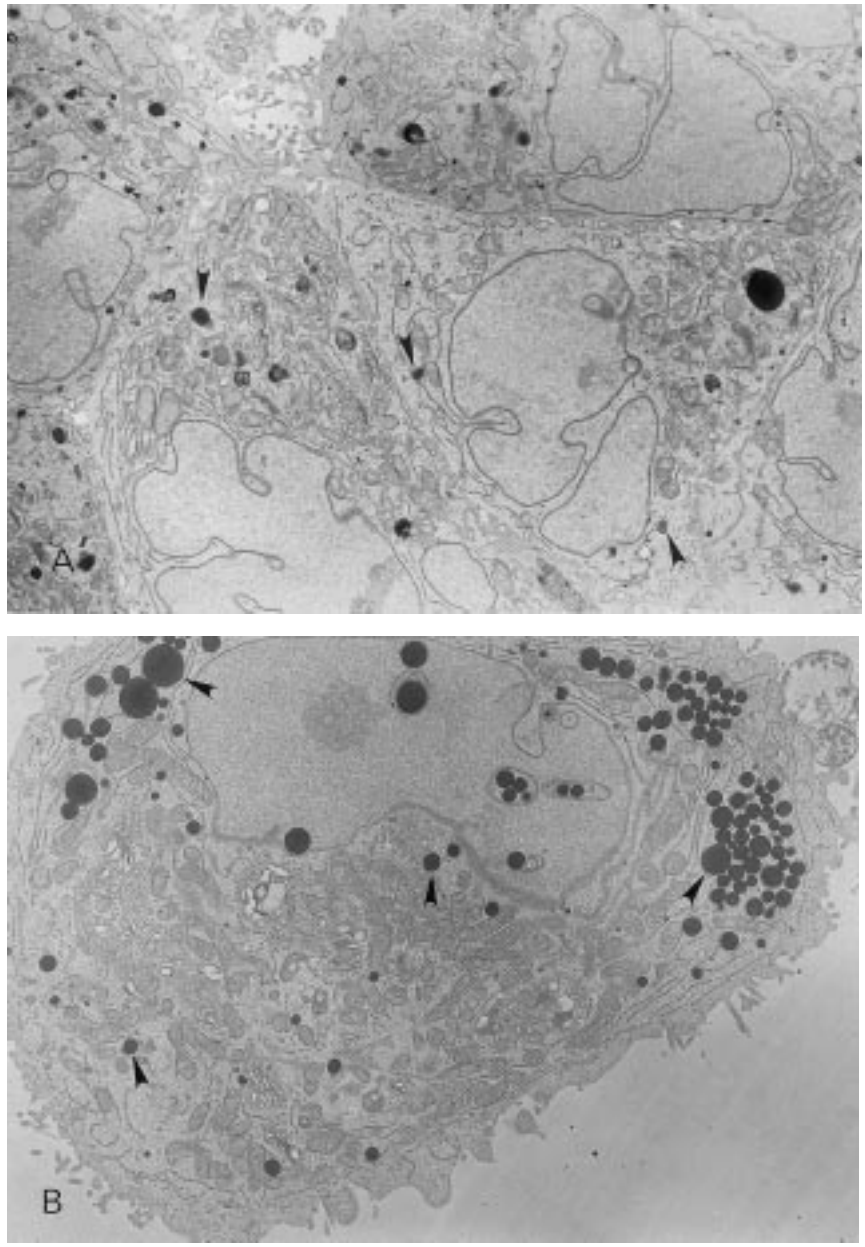

Fig. 5. (A) Microphotograph showing a cluster of untreated Hep 3B cells. Hepatoma cells contain abundant mitochondria, endoplasmic reticulum, Golgi apparatus and microvilli, the latter are interdigitated with each other. Note that a few lipide droplets (arrows) are scattered in the cytoplasm (magnification 6000 ×); (B) microphotograph showing a Hep 3B cell after 10 min incubation with 50 μM cantharidin. The cell displays similar ultrastructures to those of untreated cells except that increased lipid droplets (arrows) are concentrated in the vicinity of the nucleus (magnification 6000 ×); (C) microphotograph showing a Hep 3B cell after 30 min incubation with 50 μM cantharidin. Within this time interval, the treated cell protruded blisters (large arrows) instead of numerous microvilli. Some swollen mitochondria (M) are observed. A significant increase of glycogen particles (small arrows) is also seen around the Golgi apparatus and in the cell margin where the particles are mingled with lipid droplets (magnification 10 000 ×); (D) Microphotograph showing Hep 3B cells after 1 h incubation with 50 μM cantharidin. The treated cells display swollen endoplasmic reticulum (small arrows), perinuclear cisternae (arrows) and mitochondria (M). Note the deformed lipid droplets (large arrows) (magnification 6000 ×).

1998). The bioactive analogue of cantharidin, norcantharidin, accelerates the cell cycle progression from the G_1 phase at low concentration while inhibiting progression from the G_2/M phase (HL-60) (Liu et al., 1995). The extent of DNA fragmentation was quantified by FACS analysis, in which fragmented DNA can be detected as a small peak before the G_1 peak. After treatment with cantharidin, some apoptotic-like cells (sub-G_1) were found before the G_1 peak (Fig. 4B, C). The sub-G_1 peak indicated that a few Hep 3B cells were lyzed by cantharidin. In addition, the results of this study suggested that the action of cantharidin was cell cycle nonspecific.

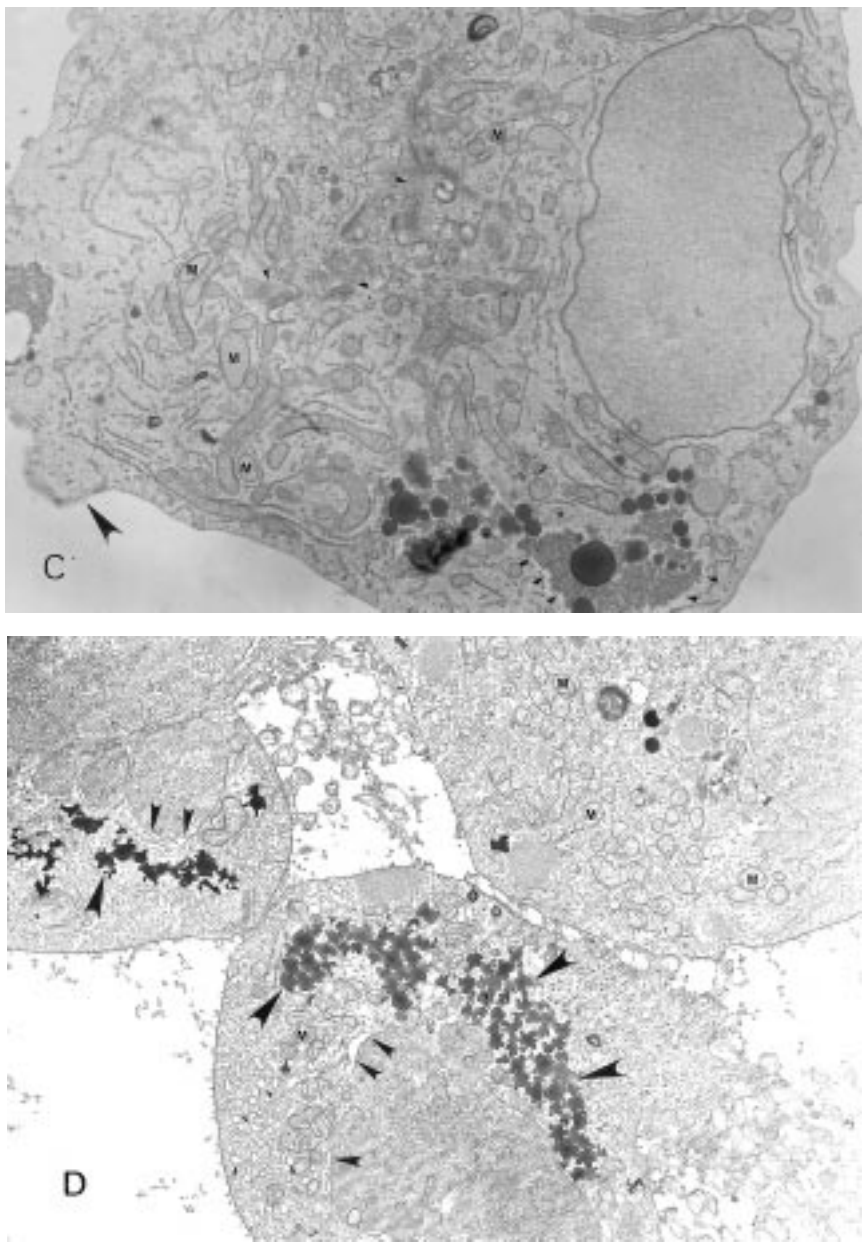

Fig. 5. (*Continued*)

Cellular injury may lead to a complex sequence of changes to structural and molecular events, frequently culminating in cell death. Many subcellular structures, such as the plasma membrane, nucleus, mitochondria, endoplasmic reticulum and lysosomes are all targets for cell toxins (Boobis et al., 1992). The ultrastructured observations in the present study revealed that Hep 3B cells induce cellular damage as early as 1 h following exposure to cantharidin and that Hep 3B cells initially displayed this damage as glycogen accumulation and swollen mitochondria, followed by hypertrophy of the endoplasmic reticulum. Coincidentally, the visual characteristics of the nuclei of treated Hep 3B cells on electron microscopy were not significantly different from those of controls. These results may support the presumption that cantharidin cannot directly inhibit nucleic acid biosynthesis. Cantharidin has been previously shown to be a strong phosphatase inhibitor, which may decrease the activities of protein phosphatase (PP1) and phosphatase 2A (PP2A) (Graziano et al., 1988; Li et al., 1993). In addition, Zhang et al. reported that cantharidin inhibited cAMP phosphodiesterase activity in hepatoma cells (Zhang et al., 1985). The ultrastructural observations in the present study of swollen mitochondria in the cantharidin-treated cells may support these alterations in enzyme activities. Damage to mitochondria in treated cells may signal the presence of an energy deficiency, particularly the loss of ATP, which may subsequently lead to functional problems involving the plasma membrane and result in acute cell death (Spungin and Friedberg, 1993). Defects in the energy system may also result in glycogen overload in hepatocytes (Wiener et al., 1968). Similar events were found in the present study including the swelling of mitochondria with glycogen concentration either around the Golgi complexes or in the peripheral cytoplasm of cells. These findings suggest that cantharidin may be a potent cytotoxic drug for some cancer cells due to its ability, to impair the mitochondrial energy pathways.

Anticancer drugs generally block the cell cycle at a specific phase. For example, vincristine inhibits the assembly of microtubles and adriamycin inhibits the DNA biosynthesis pathway. The cell cycle effects of anticancer agents are often used to predict effective combination treatments. Additive anticancer activity generally requires that two drugs have different effects on the cell cycle, indicative of different and complementary mechanisms of activity. Since cantharidin is unusual in its ability to prevent progression in all phases of the cell cycle, it follows that it may be effective when used in combination with a wide variety of standard chemotherapeutics.

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