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Antitumour imidazoacridone C-1311 induces cell death by mitotic catastrophe in human colon carcinoma cells

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Abstract

In this study, we investigated the cell death process induced by imidazoacridone C-1311 (Symadex™) in HT-29 human colon carcinoma cells which have been shown to be preferentially sensitive to this compound in experimental tumour models both in vitro and in nude mice. Compound C-1311 at the EC_{99} dose delayed progression of cells through the S phase which was followed by G2 arrest. At 48–96 h after drug exposure, an increasing fraction of cells rounded up and detached from the substratum which suggested the induction of cell death. This was confirmed by the induction of DNA fragmentation as revealed by pulse field electrophoresis and DNA strand breaks by the TUNEL assay. The dying cells had also mitotic features which were evidenced by various biochemical and morphological criteria such as activation of Cdk1 kinase, presence of the mitotic epitope MPM-2 and condensation of chromatin into mitotic chromosomes in drug-treated cells. These results show that C-1311 does not induce rapid apoptosis in HT-29 cells, instead drug exposure leads to prolonged G2 arrest followed by G2 to M transit and cell death during mitosis in the process of mitotic catastrophe.

Keywords: Imidazoacridone; C-1311; Cell cycle; G2 arrest; Cell death; Mitotic catastrophe

1. Introduction

Imidazoacridones are a group of antitumour compounds synthesized in our department which exhibit high cytotoxic and antitumour activity toward several experimental tumours, including significant activity in colorectal cancer models [1–3]. The most active compound from this group, C-1311 or Symadex™, showed potent antitumour activity, unusual pharmacokinetics properties and preferential accumulation in tumour tissue in vivo [4]. It has also been demonstrated that C-1311 does not induce free radical formation and, unlike doxorubicin and other anthracyclines, this compound is not cardiotoxic [5]. Compound C-1311 (Symadex) has recently entered phase I clinical trials for the treatment of patients with advanced solid tumours.

The mechanism of biological action of C-1311 is still under active investigation. This drug as well as other imidazoacridones bind non-covalently to DNA by intercalation [3,6,7], however, the affinity of these compounds to DNA does not correlate with their cytotoxic and antitumour activity [7]. C-1311 has been shown to inhibit the catalytic activity of DNA topoisomerase II in vitro and in tumour cells [8]. One of the most striking features of this compound is that, in contrast to classical inhibitors of topoisomerase II such as mitoxantrone and doxorubicin, C-1311 was found equally active toward cells grown in monolayer culture as well as in three dimensions as multicellular spheroids [8]. This suggests that C-1311 could be active toward tumours with high fraction of plateau phase and non-dividing cells. Other studies have shown that C-1311 undergoes enzymatic oxidation in vitro which leads to formation of reactive iminoquinone-like derivatives [9,10] and covalent DNA binding by the activated drug [11].

Previous studies have shown that biologically active imidazoacridones induce irreversible G2 arrest followed by apoptosis in murine leukaemia and human ovarian carcinoma cells [12,13]. More recent studies have shown that although C-1311 is highly active toward ovarian and sarcoma tumour cells, which are inherently resistant to

Abbreviations: PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; SDS, sodium dodecyl sulphate

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many other antitumour drugs, but this drug induced only very low levels of apoptosis in these tumours [14].

The aim of this study was to elucidate the mechanism of anti-proliferative action of C-1311 toward human colon adenocarcinoma HT-29 cells, which have been shown to be preferentially sensitive to this compound in xenografts in nude mice [5]. In particular, we were interested in events which are subsequent to G2 arrest induced by C-1311 and the actual mechanism of cell death elicited by this compound in human colon carcinoma cells. Our studies show that drug-treated cells arrest initially in G2 phase and progress from G2 to mitosis but are unable to undergo cytokinesis and die in the process resembling abortive mitosis or ''mitotic catastrophe''.

2. Materials and methods

2.1. Chemicals

Compound C-1311 (see Fig. 1, panel A for chemical structure) was synthesized by Dr. W.M. Cholody in our department. Methyl-[^{14}C]-thymidine (specific activity 52 mCi/mmol) was from Amersham Pharmacia Biotech; proteinase K was from Merck, all other reagents were from Sigma–Aldrich–Fluka.

2.2. Cell culture

HT-29 human colon adenocarcinoma cells were kindly provided by Dr. John A. Double (University of Bradford, U.K.), the media and foetal bovine serum and antibiotics were from Invitrogen. Cells were grown in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% foetal bovine serum and antibiotics (0.1 μg/ml streptomycin, 100 U/ml penicillin). The cells were maintained at 37 °C in a humidified 10% CO_2–90% air atmosphere. Under these growth conditions the cell doubling time was about 33 h.

2.3. Cytotoxicity assays

Cell survival was determined by colony formation assay. Exponential cultures were plated about 24 h before drug treatment and incubated with the drug at appropriate concentrations for 3 h. Colony forming ability was determined by seeding 1000 cells/6 cm Petri dish. Colonies were counted 14 days later after staining with Giemsa. The cloning efficiency for untreated cells was about 90%.

2.4. Morphological examinations

Cell and nuclear morphology were determined by Hoffman relief contrast and fluorescence microscopy, respectively. Cells were analysed directly in growth medium or washed twice in PBS before fixing in 1% formaldehyde for

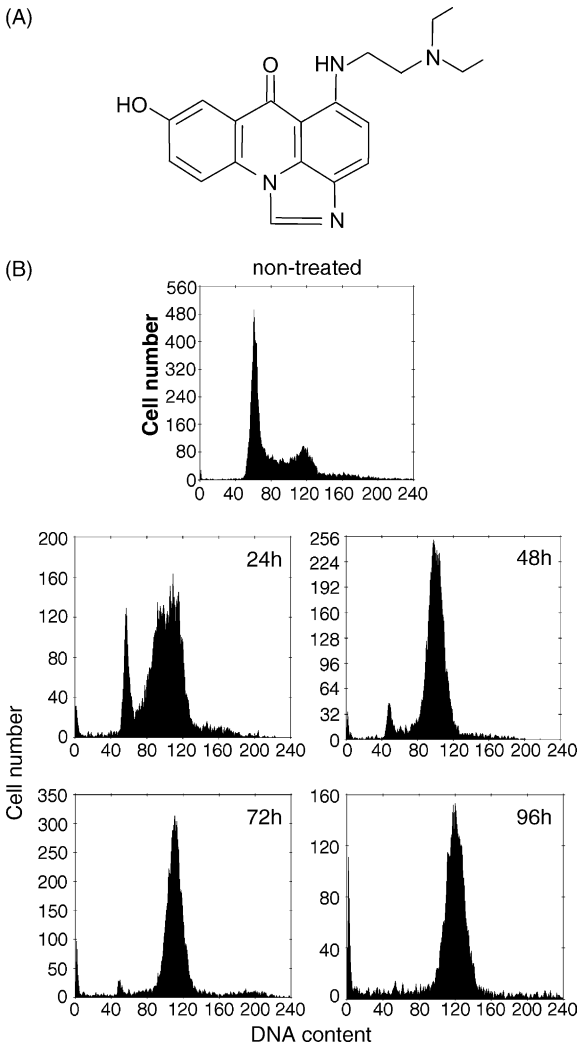

Fig. 1. Panel A: chemical structure of C-1311; panel B: cell cycle progression of HT-29 cells treated with 15 μM C-1311 for 3 h and post-incubated in drug-free medium. At indicated time periods, distribution of cells in the cell cycle was analysed by flow cytometry.

15 min and staining with DAPI (0.1 μg/ml). Photographs were taken using NIKON Eclipse TS100 inverted microscope or OLYMPUS BX60 epifluorescence microscope, photographed using AxioCam digital camera (Zeiss) and further processed by MicroImage image analysis software (Media Cybernetics). Mitotic spreads were prepared following Carnoy's fixation according to classical methods, stained with DAPI and photographed using fluorescence microscope as described above.

2.5. Flow cytometry

Distribution of cells through the cell cycle was measured by flow cytometry using the EPICS Profile II Flow Cytometer equipped with an argon laser to give 488 nm light. The cells were fixed in 70% ethanol at −20 °C, rehydrated in PBS and stained with propidium iodide (20 μg/ml) and ribonuclease A (100 μg/ml) at room temperature for 30 min. The percentage of cells in each phase of the cell

cycle was calculated by MultiPlus software (Phoenix Flow Systems).

2.6. Immunofluorescence staining

For indirect immunofluorescence staining, cells were fixed with 70% ethanol at −20 °C overnight, rehydrated in ice-cold PBS for 10 min and permeabilized in 0.25% Triton X-100/PBS for 5 min on ice. Cells were then washed with PBS and blocked in 1% BSA/PBS for 15 min at room temperature. After washing with PBS, cells were incubated for 1 h at room temperature with anti-MPM-2 (DAKO) and anti-cyclin B1 (Santa Cruz Biotechnologies) antibodies diluted at 1:100 in 0.5% BSA/PBS. Samples were washed with TBP buffer (0.2%Tween 20/0.5% BSA/PBS) and incubated with anti-mouse IgG-FITC (Amersham) and anti-rabbit IgG-Cy-5 (Jackson ImmunoResearch Labs.) for 1 h at room temperature and analyzed by flow cytometry.

2.7. TUNEL assay

Following drug treatment, cells were collected by centrifugation and fixed in 1% formaldehyde in PBS for 15 min on ice. After centrifugation the pellet was washed in PBS, re-suspended in 70% ethanol and samples were stored at −20 °C. After re-hydration in PBS, cells were labelled with terminal transferase and biotin-deoxyuridine triphosphate (dUTP) followed by staining with avidin-FITC conjugate as described [15]. DNA was stained with propidium iodide (5 μg/ml propidium iodide and 100 μg/ml ribonuclease A in PBS) at room temperature for 30 min. The red (propidium iodide) and green (fluorescein) fluorescence were measured with a Becton–Dickinson FACScan flow cytometer, the data were analysed by the MultiPlus software.

2.8. DNA fragmentation

High molecular weight DNA fragmentation was determined by inverted pulse field gel electrophoresis. Following drug treatment, cells were washed in PBS and embedded in 0.75% low melting agarose (FMC Bioproducts). Plugs were then incubated in lysis buffer (10 mM Tris–hydrochloride, pH 8, 10 mM sodium chloride, 25 mM EDTA, 0.9% sarcosyl, 0.1% SDS, 1 mg/ml proteinase K) for 24 h at 50 °C, washed three times in washing buffer (10 mM Tris–hydrochloride, pH 8, 50 mM EDTA) and stored in 50 mM EDTA, pH 8 at 4 °C until analysis. Samples, including 50–1000 kbp lambda DNA marker (BioRad), were analysed in a 1% FastLane agarose gel (FMC Bioproducts) in 0.5× TBE buffer (45 mM Tris–borate, 1 mM EDTA, pH 8.3) with temperature maintained at 6 °C using FIGE Mapper system (BioRad). The pulse-wave switcher was programmed to provide initial 9 s forward and 3 s reverse pulses, with linear ramp 3:1 and a constant voltage of 180 V throughout 12 h. Gels were stained and photographed under UV illumination.

2.9. Quantitation of DNA fragmentation by filter-binding assay

Cells were labeled with [^{14}C]-thymidine (0.05 μCi/ml, 48 h) and chased in radiolabel-free medium for 6 h. Approximately 5×10^5 cells suspended in 5 ml ice-cold PBS were applied onto Matricel filters (Gelman Sciences) and washed twice with 5 ml PBS. DNA fragmentation was determined by the filter-binding assay as described [16].

2.10. Western blot analysis

Cells were lysed in ice-cold RIPA buffer (50 mM Tris–hydrochloride, pH 7.5, 150 mM sodium chloride, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) containing protease inhibitor coctail (Roche Molecular Biochemicals) and phosphatase inhibitors (5 mM sodium orthovanadate, 50 mM sodium fluoride, 10 mM β-glycerophosphate) for 15 min on ice. Fifty micrograms of protein was loaded on each lane, separated on SDS-PAGE (10–12% polyacrylamide gel) and transferred onto polyvinylidene difluoride membranes (BioRad). After blocking with TBS buffer (10 mM Tris–hydrochloride, pH 7.5, 150 mM sodium chloride) containing 0.2% Tween 20 and 5% non fat milk, the blots were incubated with primary antibodies: anti-cdc2, anti-phosphotyrosine, anti-cyclin A, anti-cyclin B1 and anti-actin (all from Santa Cruz Biotechnologies) diluted at 1:100 in TBST (TBS containing 0.2% Tween 20 and 0.5% milk) at room temperature for 1–4 h. The blots were then washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Labs.) diluted at 1:40,000 in TBST) for 1 h at room temperature. After additional washes in TBST, the protein bands were detected by the ECL enhanced chemiluminescence system (Amersham) using Hyperfilm-ECL film.

3. Results

3.1. Cells treated with C-1311 arrest in G2

HT-29 cells treated with the EC_{99} concentration of the drug for 3 h and post-incubated in drug-free medium showed a transient delay in S phase of the cell cycle, with the maximal S-phase fraction after 24–48 h post-incubation, followed by progressive accumulation of cells in G2/M at 72–96 h (Fig. 1, panel B). Longer incubation times produced severe cell cycle perturbation precluding its analysis by flow cytometry due to the presence of dying or dead cells. Similar effect on cell cycle progression of HT-29 cells was induced by equitoxic dose of C-1311 (3 μM) with continuous exposure to the drug, which also

produced a delayed progression through the S-phase and G2/M arrest, with the maximal fraction of G2/M cells after 72 h drug exposure (not shown). Analysis of biochemical markers of cell cycle progression by Western blotting showed rapid accumulation of both cyclin A and cyclin B1 and the appearance of slow migrating, phosphorylated forms of cdc2 protein at 24–48 h after drug treatment (Fig. 2). Phosphorylated forms of cdc2 protein progressively disappeared between 72 and 120 h which was accompanied by the decrease in cyclin B1 content. Interestingly, cyclin A which accumulated during 24–48 h post-incubation but only slightly decreased at 72–120 h after drug treatment. These results show that drug-treated cells accumulated in G2 phase with high cyclin A and B1 content as well as with inactive cdk1 kinase.

3.2. Drug-treated HT-29 cells enter mitosis

Western blot analysis suggested that cells treated with C-1311 initially arrested in G2 but then progressed to mitosis. To further confirm these results, we followed changes in cell morphology by light microscopy and we observed increased fraction of cells which rounded up at post-incubation times longer than 48 h (Fig. 3). In parallel, mitotic index determination showed that a number of the mitotic cells increased over time of post-incubation (Fig. 4). Analysis by fluorescence microscopy of chromosome spreads from drug-treated cells have shown that in cells incubated for more than 72 h chromosomes were frequently only partially condensed, with a punctuated pattern of condensed and undercondensed chromatin (Fig. 5, panel A). Occasionally, cells with abnormal number of chromosomes per cell and with heavily fragmented or abnormally elongated chromosomes were observed (Fig. 5, panel B).

Compound C-1311 is a potent inhibitor of topoisomerase II [8] and this enzyme is one of the factors essential for chromosome condensation [17]. For this reason, it is difficult to get an accurate estimate of the mitotic fraction in cells treated with topoisomerase II inhibitors using classical mitotic spread technique, which relies on the visualization of highly condensed chromosomes.

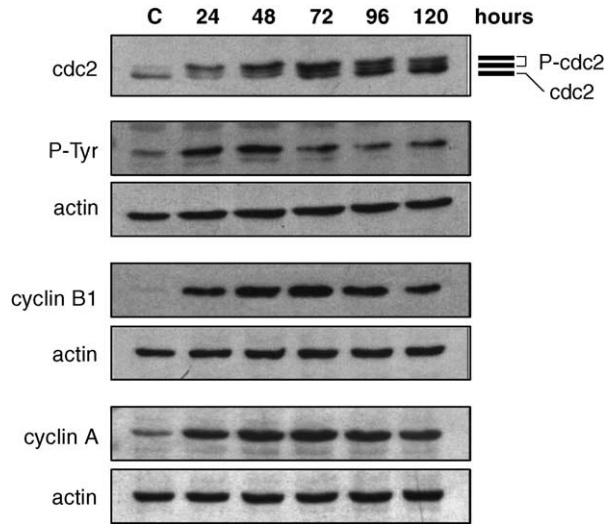

Fig. 2. Western blot analysis of cdc2, cyclin A and B expression in HT-29 cells treated with 15 μM C-1311 for 3 h and post-incubated in drug-free medium for the time indicated.

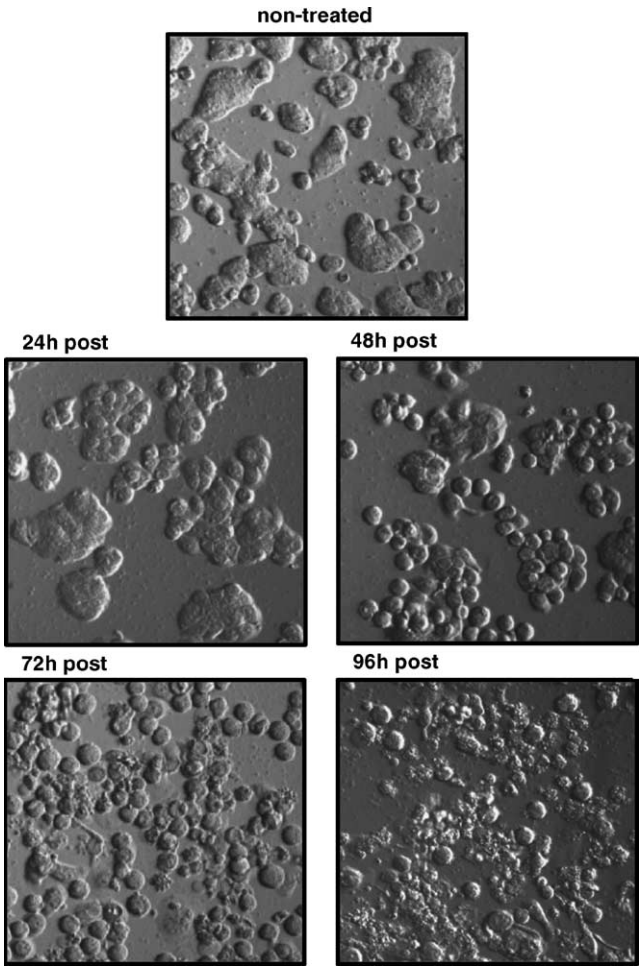

Fig. 3. Cellular morphology of HT-29 cells treated with 15 μM C-1311 for 3 h and post-incubated in drug-free medium. Cells were analysed by phase contrast microscopy.

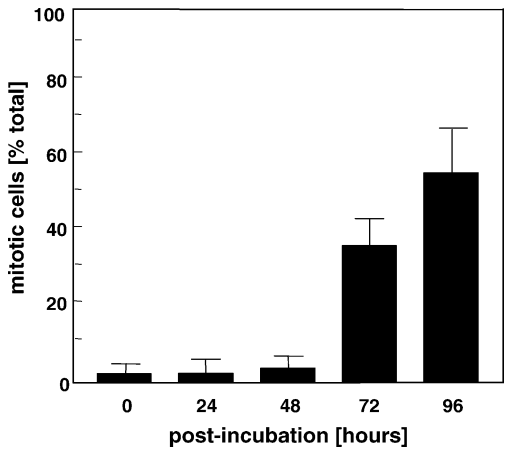

Fig. 4. Mitotic index of HT-29 cells treated with 15 μM C-1311 for 3 h and post-incubated in drug-free medium for the time indicated.

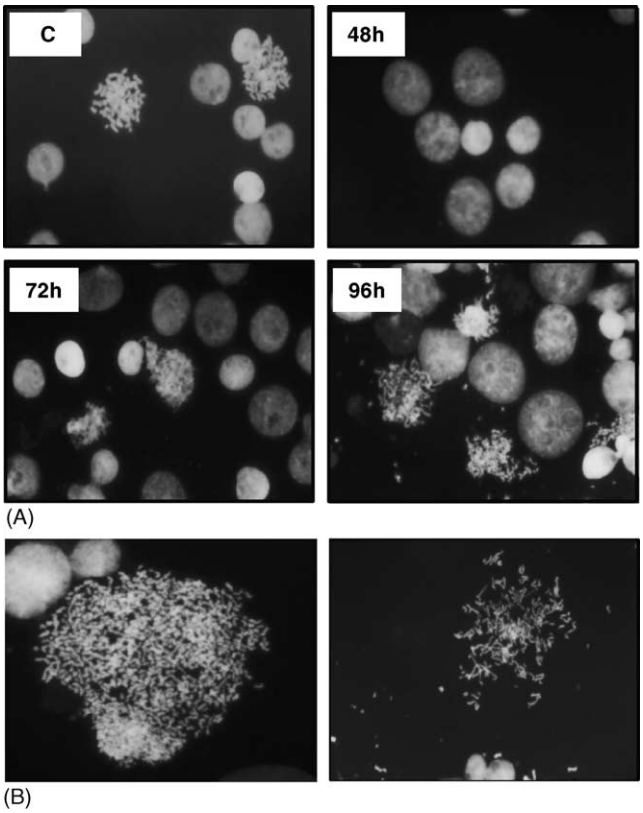

Fig. 5. Nuclear morphology of HT-29 cells treated with the drug and post-incubated in drug-free medium for the time indicated. Cells were fixed, stained with DAPI and analysed by fluorescence microscopy: panel A: nuclear morphology of drug-treated cells at different time periods after drug-treatment; panel B: chromosome spreads from HT-29 cells treated with the drug and post-incubated for 96 h showing a polyploid cell (left) and a cell with fragmented chromosomes (right) undergoing mitotic cell death. Original magnification 400× (panel A) and 1000× (panel B).

Therefore, to confirm our mitotic index data we used double-epitope immunostaining assay with anti-cyclin B1 and MPM-2 directed antibodies followed by biparametric flow cytometry analysis. The MPM-2 phosphoepitope is characteristic for mitosis and is present in cells from early prophase to anaphase [18]. Using this assay, we could quantitatively determine fractions of interphase-G2 cells (MPM-2-negative) and mitotic cells (cyclin B1-positive and MPM-2-positive). As a reference compound, we used nocodazole (50 ng/ml), a microtubule inhibitor, which blocks cells in prometaphase where cells have both high levels of cyclin B1 and the MPM-2 epitope (Fig. 6, panel A, region B). For cells treated with C-1311, there was an increase in the fraction of cyclin B1-positive cells at longer post-incubation times with the maximal fraction of about 80% after 96 h after drug treatment (Fig. 6, panel B). Interestingly, there was also an increase of MPM-2 positive cells with a peak value of 35% total cell population at 96 h. The levels of cyclin B1 decreased about two-fold between 72 and 96 h post-incubation (Fig. 6, arrows) whereas drug-treated cells kept the MPM-2 signal relatively unchanged during this time period.

3.3. Cells treated with C-1311 die during mitosis

Flow cytometry analysis of cells treated with C-1311 suggested that after post-incubation times longer that 72–96 h following drug treatment cell death was induced. This was also evident from the analysis of cell morphology which showed that starting from 96 h post-incubation, the majority of cells detached from the substratum (Fig. 3). We also analysed the integrity of DNA in drug-treated cells by pulse field gel electrophoresis and found increasing levels of high molecular DNA fragmentation (50–300 kbp fragments) with increasing time after drug treatment (Fig. 7, upper panel). The kinetics of DNA fragmentation analysed by the filter elution assay (Fig. 7, lower panel) showed a gradual increase in DNA fragmentation reaching about 50% of the total cellular DNA after 120 h after drug treatment.

We observed that induction of cell death and DNA fragmentation in drug-treated HT-29 cells coincided with the appearance of mitotic cells. This suggested to us that cell death occurs in HT-29 cells treated with C-1311 in a cell cycle-specific manner. To clarify this point, the TUNEL assay was used, which allows identification the position of cells with fragmented DNA in the cell cycle. Biparametric flow cytometry analysis for DNA fragmentation and DNA content showed that cells which were stained positive for DNA fragmentation originated almost exclusively from the G2/M region (Fig. 8, upper panel). Quantitative analysis showed an increased fraction of cells with fragmented DNA with increasing post-incubation time after drug treatment which reached about 25% of the total DNA after 96 h (Fig. 8, lower panel). Interestingly, HT-29 cells which entered mitosis following G2 arrest induced by C-1311 did not resume normal cell proliferation even after two weeks of post-incubation in drug-free medium. Rather, cells gradually lost viability and eventually no viable cells were observed in drug-treated cell population even after prolonged (several weeks) incubation with occasional change of the growth medium (not shown).

4. Discussion

The aim of this study was to elucidate biological effects which are subsequent to G2 arrest induced by an anti-tumour imidazoacridone C-1311 (Symadex) in HT-29 human adenocarcinoma cells. We also wanted to clarify the role of apoptosis in cell killing effect induced by C-1311 in colon carcinoma cells. We explicitly chose HT-29 cells for our study since C-1311 showed preferential activity toward colon tumours both in cell culture and in tumour-bearing mice [3,4]. Our study showed that human colon adenocarcinoma HT-29 cells exposed to the EC_{99} dose of C-1311 undergo prolonged but transient G2 arrest followed by G2 to M progression and cell death during cell division.

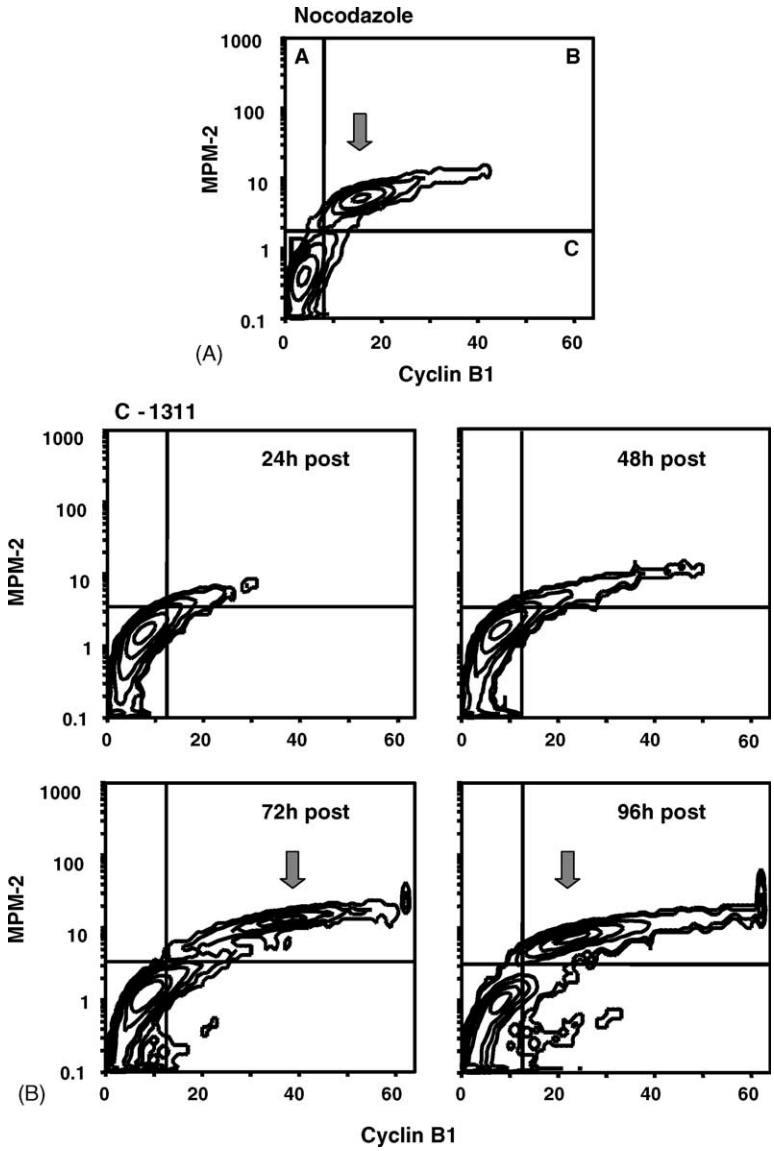

Fig. 6. Changes in cyclin B1 expression and the presence of the mitotic MPM-2 epitope in drug-treated HT-29 cells as revealed by immunofluorescence staining followed by two parameter flow cytometry: panel A: cells treated with 50 ng/ml nocodazole for 12 h; panel B: cells treated with 15 μM C-1311 for 3 h and post-inucubated in drug-free medium for the time indicated. Arrows show cell populations positive for the presence of MPM-2 epitope (prophase to anaphase) and with high levels of cyclin B1 (G2 to telophase).

Progression from G2 to mitosis is controlled by a set of surveillance mechanisms called cell cycle checkpoints which are activated by different stimuli such as DNA damage and aberrant functioning of the mitotic spindle. Activation of these checkpoints leads to growth arrest which is believed to provide the cell additional time to repair the potentially lethal lesions and thereby renders cells more resistant to the cytotoxic effects of anticancer drugs [19]. In human tumours, the G1 checkpoint is frequently inactivated due to non-functional p53 protein. The G2 checkpoint functioning depends only partially on p53 [20], therefore, many human tumour cells entirely depend on the functioning of the G2 checkpoint when exposed to DNA damaging agents.

In previous studies, no signs of apoptosis were observed in HT-29 cells treated with C-1311 over a 24 h continuous drug treatment [21]. More recent studies with ovarian and osteosarcoma tumour cells have also shown that at the IC_{50} concentration of C-1311, only a minor fraction of cells underwent apoptosis after 72 h drug treatment [14]. These studies confirm our observations that apoptosis is not a major mechanism of cell killing induced by C-1311 in HT-29 cells. An alternative mechanism of cell death induced by C-1311 in HT-29 cells has been suggested, which is drug-induced lysosomal rupture followed by cell autolysis [21]. However, during our studies we did not observe any major changes in the morphology of lysosomes in HT-29 cells treated with C-1311 (not shown) and this difference is most probably related to a pulse treatment of cells with the drug (3 h) instead of the continuous exposure used in previous studies [21].

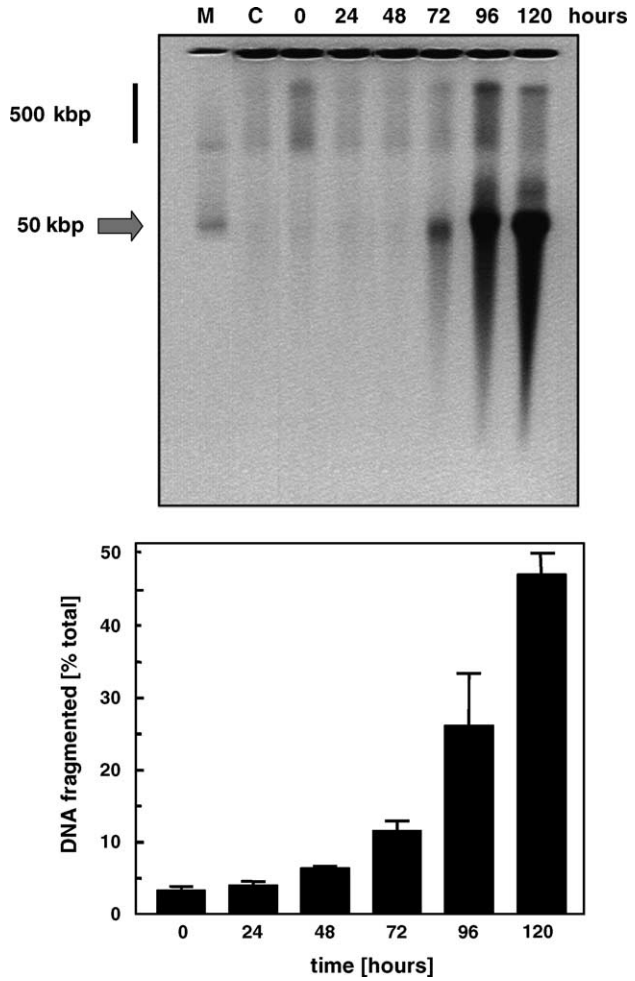

Fig. 7. DNA fragmentation in HT-29 cells treated with 15 μM C-1311 for 3 h and post-incubated in drug-free medium. Panel A: cells were embedded in low melting agarose plugs, lysed and analysed by pulse field gel electrophoresis: lane M, DNA marker; lane C, non-treated cells; lane 0, cells treated with the drug for 3 h; lanes 24, 48, 72, 96 and 120, cells treated with the drug and post-incubated for the time indicated (h). Panel B: levels of DNA fragmentation in drug-treated cells as determined by the filter-binding assay.

Previous studies which have shown that C-1311 arrests cell cycle progression of tumour cells in G2 phase and cells die from the apparent irreversible G2 block by apoptosis in murine leukaemia L1210 [12] and human cervical carcinoma HeLa S3 cells [13]. This is in partial contradiction with our results obtained with C-1311-treated HT-29 cells (this study). However, it should be pointed out that in these earlier studies cell cycle analysis was performed using a detergent-based method for nuclei isolation [22], where cell nuclei, instead of whole cells, are analyzed by flow cytometry. In this method, mitotic cells do not contribute to the G2/M peak since cells in mitosis do not contain discrete nuclei and are completely dissolved during detergent-based preparation of the nuclear suspension. The same could apply to dying cells which may be, at least partially, lost in lysing buffer. It follows that in these earlier studies some of the cell cycle- and cell death-related effects might have been left unrevealed, especially if drug-treated cells progressed from G2 to M and/or G2 arrest was accompanied by cell death. In our studies, we analyzed by flow cytometry HT-29 cells and not cell nuclei which could explain this apparent discrepancy between our results (this study) and the results of others [12,13].

It is intriguing that HT-29 cells treated with C-1311 arrested initially in G2 phase and then progressed to mitosis and died during cell division. It is possible that G2 arrest induced by the drug cannot be maintained in HT-29 cells for prolonged time periods by DNA damage checkpoint mechanisms and cells eventually progress from G2 to mitosis. It should be noted that C-1311 induces only low levels of DNA damage compared to classical topoisomerase II inhibitors, such as m-AMSA [8]. Therefore, it is possible that such low levels of DNA damage are unable to effectively activate the DNA damage checkpoint in HT-29 cells. Another possibility is adaptation of cells to DNA-damage checkpoint in G2 which has been described both in yeast and human colon tumour cells treated with different antitumour drugs and γ-irradiation [23,24]. Recent studies suggest that in cells with non-functional p53, G2 arrest following DNA damage could not be maintained [25]. HT-29 cells express high levels of mutated p53 protein [26] but recent studies showed that the functionality of p53 seems not to be a determining factor of cellular susceptibility to C-1311 of different solid tumours in vitro [14].

Another question is why HT-29 cells which escape G2 arrest induced by C-1311 do not complete mitosis, instead, cells fail to divide and undergo cell death during cell division. The presence of mitotic chromosomes and MPM-2 epitopes in drug-treated HT-29 cells suggests that cells progress from G2 at least until metaphase–anaphase transition, therefore, cells apparently die while attempting segregation of damaged and/or catenated chromosomes in the process resembling abortive mitosis or mitotic catastrophe. This scenario has already been proposed for irradiated cells which did not die until cell division [27]. Mitotic catastrophe has also been observed in cells exposed to DNA damaging agents in which functioning of DNA checkpoint has been abrogated by checkpoint modulators or genetic changes [28–31]. Another interesting possibility is that spindle checkpoint or DNA structure checkpoint could be activated by DNA damage [32]. If this also can happen in HT-29 cells, cells treated with C-1311 would progress into mitosis following initial G2 arrest and stop before the metaphase–anaphase transition due to activation of the spindle checkpoint by unrepaired DNA damage. HT-29 cells have functional spindle checkpoint and undergo mitotic arrest following spindle damage [33].

In conclusion, we show here that antitumour agent, Symadex or C-1311, with high activity toward colon tumours induces prolonged G2 arrest in HT-29 colon adenocarcinoma cells. This G2 arrest induced in drug-treated cells is followed by a transit to mitosis and consequently cell death in the process resembling abortive mitosis or mitotic

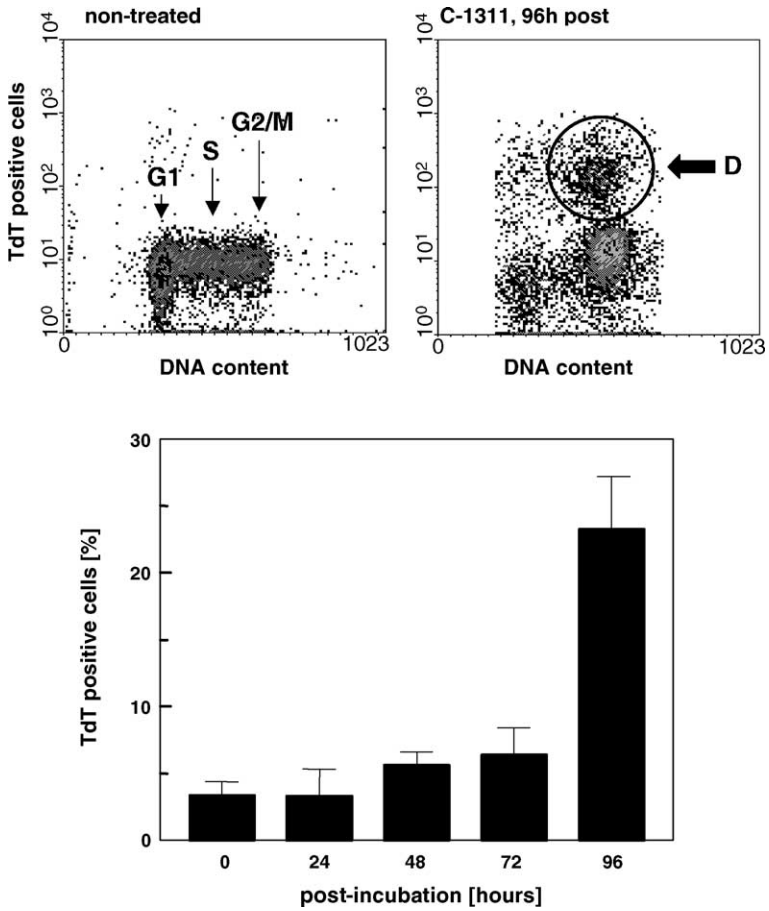

Fig. 8. Cell cycle specific induction of cell death by C-1311 in HT-29 cells as determined by the TUNEL assay. Upper panel: apoptotic cells were identified after the fragmented DNA was labeled with biotin-dUTP an avidin-FITC, all cells were counterstained with propidium iodide to determine their position in the cell cycle and analysed by two parameter flow cytometry. Arrow D shows the dying cell population in G2/M region. Lower panel: quantitative representation of the flow cytometry data with fractions of the TdT-positive cells at different post-incubation time periods.

catastrophe. Further studies are under way aimed at characterization of the molecular mechanism(s) of mitotic cell death induced by C-1311 in HT-29 cells.

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