MLH1-deficient tumor cells are resistant to lipoplatin, but retain sensitivity to lipoxal

André Fedier[a], Cédric Poyet[a], Daniele Perucchini[a], Teni Boulikas[b] and Daniel Fink[a]

Lipoplatin, currently under phase III evaluation, is a novel liposomal cisplatin formulation highly effective against cancers. Lipoplatin has eliminated or reduced the systemic toxicity frequently seen for cisplatin. The objective of the present study was to determine whether the cytotoxic effect of lipoplatin is dependent on the functional integrity of DNA mismatch repair (MMR), a post-replicative DNA repair machinery implicated in cell cycle control and apoptosis. Clonogenic data revealed a significant ($P<0.05$) 2-fold resistance to lipoplatin of HCT116 human colorectal adenocarcinoma cells lacking MLH1, one of five proteins crucial to MMR function, as compared to MLH1-expressing HCT116 cells. In addition, MLH1-deficient cells were at least 3-fold less susceptible to apoptosis (DNA fragmentation) than MLH1-proficient cells. However, proteolytic processing of caspase-3, caspase-7 and poly(ADP-ribose)polymerase-1 following lipoplatin treatment was comparable in MLH1-deficient cells and -proficient cells. Furthermore, MLH1-deficient cells retained the ability to attenuate cell cycle progression past the G_2/M checkpoint following lipoplatin treatment. In conclusion, our results indicate that the lipoplatin-sensitive phenotype of MLH1-proficient cells correlated with increased apoptosis which may occur via caspase-independent pathways. They also suggest that the integrity of MMR function is a relevant determinant accounting for the cytotoxicity of lipoplatin. However, this does not seem to apply to lipoxal, a novel liposomal formulation of oxaliplatin, because MLH1-deficient cells were as sensitive to lipoxal as MLH1-proficient cells. *Anti-Cancer Drugs* 17:315–323 © 2006 Lippincott Williams & Wilkins.

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[a]Department of Gynecology, University Hospital of Zurich, Zurich, Switzerland and [b]Regulon Inc., Mountain View, California, USA

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Correspondence to A. Fedier, Department of Gynecology, University Hospital of Zurich, Frauenklinikstrasse 10, 8091 Zurich, Switzerland
Tel: +41 44 255 5375; fax: +41 44 255 4553;
e-mail: andre.fedier@usz.ch

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Introduction

Cisplatin is one of the most widely used and most effective cytotoxic agents in the treatment of cancers, including those of the lung, head and neck, bladder, ovary, cervix, colon, and prostate. The anti-tumor properties of cisplatin are attributed to the kinetics of its chloride ligand displacement reactions leading to DNA cross-linking activities. The major mechanism of cisplatin cytotoxicity arises from its ability to induce monofunctional DNA adducts as well as intrastrand and interstrand crosslinks. Recognition of cisplatin lesions by high-mobility-group (HMG) non-histones, and binding of HMG domain proteins to cisplatin-modified DNA and subsequent activation of apoptosis pathways have been postulated to mediate the anti-tumor properties of cisplatin [1–5].

However, the continued clinical use of cisplatin revealed severe adverse reactions such as renal toxicity, ototoxicity, peripheral neuropathy and gastrointestinal toxicity [5]. Lipoplatin, a liposomal formulation of cisplatin, was developed in order to reduce the systemic toxicity of cisplatin while simultaneously improving the targeting of the drug to the primary tumor and to metastases. Lipoplatin consists of liposome particles of an average size of 110 nm with a lipid-to-cisplatin ratio of 10.24 mg lipid/mg cisplatin [6].

Preclinical studies in mice and rats have shown that lipoplatin has high therapeutic efficacy, but had reduced nephrotoxicity as compared to those animals injected with cisplatin [6,7]. In a phase I study involving 27 patients and dose escalation to 125 mg/m^2, grades 1 and 2 gastrointestinal tract and hematological toxicities were detected at the highest doses, but no nephrotoxicity or neurotoxicity was observed. The half-life of lipoplatin was 60–117 h depending on the dose, compared to 6 h for cisplatin [8]. Intravenous infusion of lipoplatin resulted in an average 10–50 times higher accumulation of platinum in tumor tissue in four independent patient cases that underwent lipoplatin infusion followed by a pre-scheduled surgery about 20 h later, as compared with

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the adjacent normal tissue specimens, with the most effective targeting in colon cancer tissue (200-fold) as compared to normal colon tissue [9].

Cisplatin resistance is an important aspect in cancer treatment. Several activities have been identified to account for cisplatin resistance in tumor cells [10,11]. A defect in DNA mismatch repair (MMR) has been proposed to be another contributor to resistance to cisplatin and other chemotherapeutic agents [12–15]. MMR is a post-replicative DNA repair machinery that not only recognizes and corrects DNA biosynthetic errors occurring during DNA replication [16,17], but also activates cell cycle checkpoints and initiates apoptosis in the presence of damaged DNA [18,19]. The role of MMR defects in drug resistance and the potential clinical impact have been supported by clinical studies [20,21]. Another hallmark of MMR defects, arising through mutations in the MMR genes (e.g. *MLH1*, *MSH2* and *MSH6*) or through hypermethylation of the *MLH1* promoter, is the potential to predispose cells to carcinogenesis [22].

The therapeutic efficacy in mouse xenografts with breast and prostate human tumors after treatment of the xenografts with lipoplatin has previously been shown to correlate with apoptosis [6]. In the present study we investigated whether lipoplatin-induced apoptosis and checkpoint activation, and hence the sensitivity of tumor cells to this drug, is dependent on the integrity of MMR function. Our observations for lipoplatin are discussed with respect to lipoxal, a very recently developed liposomal formulation oxaliplatin, and to the corresponding naked compounds.

Materials and methods

Cell culture and drugs

The following two sublines, derived from the MLH1-deficient human colorectal adenocarcinoma cell line HCT116 (ATCC CCL 247) containing a hemizygous mutation in the *MLH1* gene and hence resulting in a truncated, non-functional protein [23], were used: a subline complemented with chromosome 3 carrying the wild-type gene for human *MLH1* (clone HCT116/3-6, identified here as HCT116 + ch3) and, for reasons of chromosome balance, a subline complemented with chromosome 2 (clone HCT116/2-1, identified here as HCT116 + ch2). The chromosome-complemented HCT116 + ch3 subline is competent in MMR, while the HCT116 + ch2 subline is incompetent. Both sublines were maintained in Iscove's modified Dulbecco's medium (Invitrogen, Basel, Switzerland) supplemented with 10% heat-inactivated FBS (Oxoid, Basel, Switzerland) and geneticin (400 µg/ml) (Invitrogen). It is generally acknowledged that the chromosome complementation does not spoil the effects of MMR on drug sensitivity, although the extent of possible effects from the introduction of an extra chromosome is not fully clear. The presence or absence of MLH1 protein in the respective sublines is routinely checked in the laboratory by immunoblotting, as is the sensitivity of the sublines to the alkylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to which the MLH1-deficient HCT116 + ch2 subline is resistant [24]. The growth rate of the two sublines was comparable (data not shown). When seeded sparsely on standard tissue culture plates, the cell lines form well-defined individual colonies. Lipoplatin, a liposomal formulation of cisplatin, and lipoxal, a liposomal formulation of oxaliplatin, were provided by Regulon (Mountain View, California, USA). Aliquots were stored at 4°C.

Cytotoxicity assays

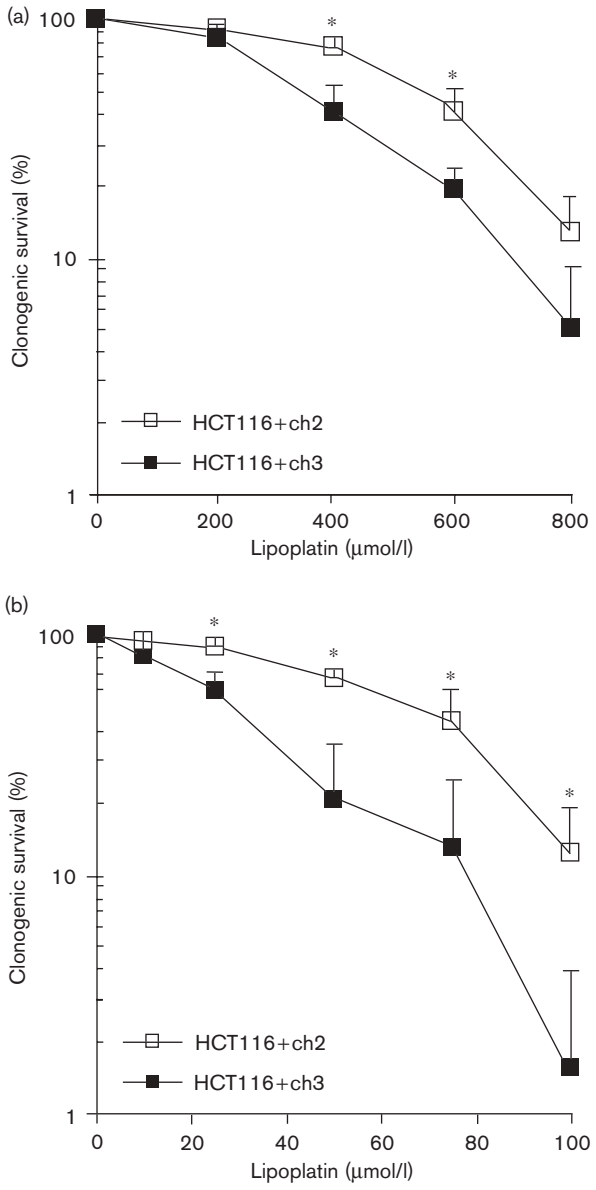
Drug sensitivity was determined by means of the clonogenic assay. Five hundred cells were plated into 60-mm cell culture dishes. Lipoplatin or lipoxal was added after 24 h and the cells were further incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 1, 6 or 24 h. The drug-containing medium was exchanged for drug-free medium, followed by incubation for another 7 days to allow colony formation. Cells were fixed with 25% acetic acid in ethanol and stained with Giemsa. Colonies of at least 50 cells were scored visually. Each experiment was performed a minimum of 3 times using triplicate cultures for each drug concentration. The logarithm of relative colony formation was plotted against the drug concentration. The IC_{50} value was estimated by linear interpolation of the logarithmically transformed relative plating efficiencies. Cell killing on the basis of plasma membrane disruption was determined by means of Trypan blue inclusion. Cells (150 000) were grown to 60% confluence in 35-mm culture dishes and incubated without or with lipoplatin at various concentrations for 1, 24 or 72 h. An exchange for drug-free medium was performed where appropriate, i.e. after 1 or 24 h post-drug addition. At 72 h after drug addition, floating and adherent cells were collected and subjected to incubation in a Trypan blue solution (0.1% final concentration) for 2 min. The proportions of Trypan blue-excluding (negative) and -including (positive) cells were determined with a hematocytometer.

Flow cytometry

Flow cytometry was performed to study the effects of drug exposure on DNA fragmentation (a measure for apoptosis) and cell cycle progression. Cells (500 000) were grown to 60% confluence in 60-mm dishes and treated with various concentrations of lipoplatin for 6 or 24 h, followed by exchange for drug-free medium and further incubation for the period of time desired. Adherent and floating cells were collected, washed in PBS, fixed with ice-cold 70% ethanol and stored at 4°C until further use. Cleavage of genomic DNA during

apoptosis yields DNA strand breaks and fragmented DNA. These are labeled by TUNEL at free 3′-OH termini with modified nucleotides in an enzymatic reaction in a template-independent manner. Fluorescein labels incorporated in nucleotide polymers are quantified by flow cytometry. After removal of the ethanol by centrifugation, cells were resuspended in the TUNEL reaction mixture, incubated at 37°C for 2 h according to the manufacturer's protocol (*In Situ* Cell Death Detection Kit, Fluorescein; Roche, Basel, Switzerland) and analyzed by flow cytometry (Epics Elite, Beckmann-Coulter, Hialeah, Florida, USA). For cell cycle analysis, the ethanol was removed by centrifugation. Cells were washed in PBS and stained in 1 ml of propidium iodide staining solution (50 µg/ml propidium iodide and 100 U/ml RNase A in PBS) by incubation at room temperature for 45 min in the dark and then washed in PBS. Samples were analyzed for their DNA content by flow cytometry (Epics Elite) and the percentage of cells in each phase was determined using the MultiCycle for Windows Software (Phoenix Flow Systems, San Diego, CA).

Fig. 1

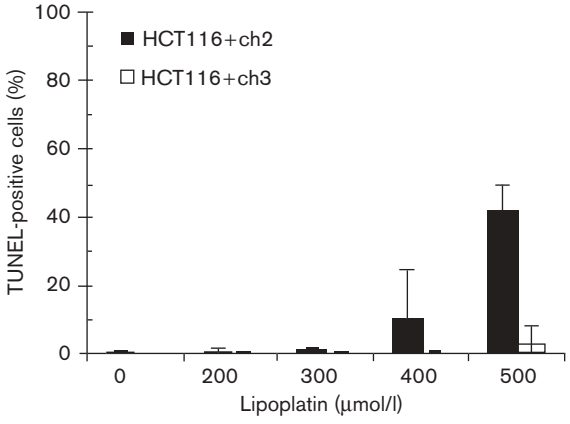


Clonogenic survival of MLH1-proficient HCT116 + ch3 (■) and MLH1-deficient HCT116 + ch2 (□) cells in response to lipoplatin treatment. Cells were treated with lipoplatin for 1 (a) or 24 h (b), further incubated to allow colony formation, and stained with Giemsa on day 7 post-lipoplatin addition. Each data point represents the mean ± SD of four independent experiments. Values with $P < 0.05$ are indicated (*).

Immunoblot analysis

Immunoblot analysis was performed to determine lipoplatin-induced apoptosis on the basis of proteolytic cleavage of caspase-3, caspase-7, and poly(ADP-ribose)-polymerase-1 (PARP-1). Cells (500 000) were grown to 60% confluence in 25-cm^2 tissue culture flasks and then treated with lipoplatin for 24 h. Cells were collected 48 or 72 h after drug addition, washed in PBS and prepared for immunoblot analysis following standard protocols. Samples of 20 µg protein were separated using 10% SDS–PAGE, followed by blotting onto a PVDF membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The full-length 32-kDa caspase-3 and 34-kDa caspase-7 precursors, the full-length 116-kDa PARP-1 precursor, and the respective proteolytic fragments were detected with the respective rabbit polyclonal antibodies (sc-7148; Santa Cruz Biotechnology, Santa Cruz, California, USA;

Fig. 2



Dependence of the extent of DNA fragmentation (TUNEL) induced by lipoplatin on the presence or absence of MLH1. Cells were treated with various concentrations of lipoplatin for 24 h, and collected for TUNEL staining and flow cytometry 72 h post-lipoplatin addition. Columns: proportion of TUNEL-positive MLH1-proficient HCT116 + ch3 (black) and MLH1-deficient HCT116 + ch2 (white) cells as a function of lipoplatin concentrations, presented as the mean ± SD of two independent experiments.

ALX-210-812 and ALX-210-302; Alexis Biochemicals, Lausen, Switzerland). Horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (sc-2030, Santa Cruz Biotechnology) was used as a secondary antibody. β-Actin, detected by monoclonal anti-β-actin (A5441; Sigma, Buchs, Switzerland) and HRP-conjugated anti-mouse antibody (M15345; Transduction Laboratories, Lexington, Kentucky, USA), served as the sample loading control. The complexes were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech). Quantitative analysis of the complexes (intensity on the autoradiograph) was performed by densitometry (normalized against β-actin) using Scion Image 4.01 Win software (Scion, Frederick, Maryland, USA).

Statistical analysis

The means ± SD were calculated. $P < 0.05$ was considered statistically significant (two-tailed paired Student's *t*-test).

Results

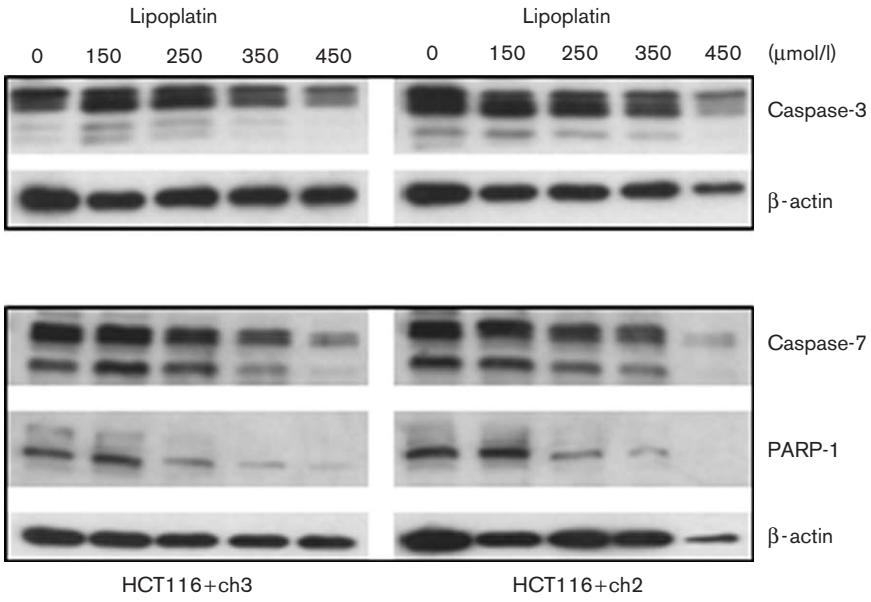
MHL1-deficiency and sensitivity to lipoplatin

Clonogenic data showed that MLH1-deficient HCT116 + ch2 cells were 1.6-fold less sensitive to a 1-h treatment and 2.3-fold less sensitive to a 24-h treatment with lipoplatin than MLH1-proficient HCT116 + ch3 cells. The IC_{50} values were 556 ± 66 versus 359 ± 39 μmol/l, $P = 0.009$ (Fig. 1a) and 70 ± 11 versus 38 ± 8 μmol/l, $P = 0.002$ (Fig. 1b), respectively. Thus, the cytostatic effect of lipoplatin is dependent on the presence of MLH1.

MLH1 deficiency and susceptibility to apoptosis

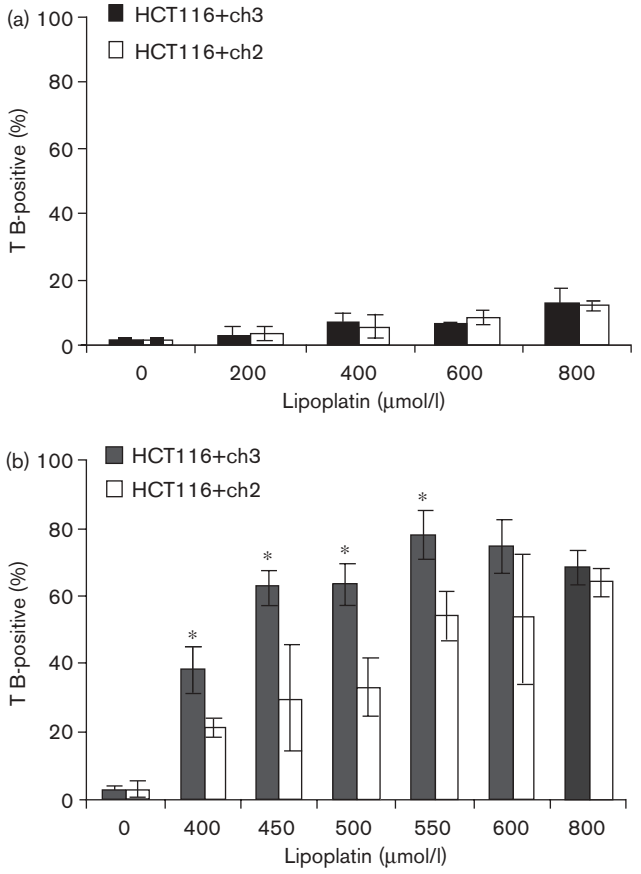
It was determined whether the reduced sensitivity of MLH1-deficient cells to lipoplatin observed in the clonogenic assay was paralleled by reduced apoptosis. Susceptibility to apoptosis was measured by DNA fragmentation (TUNEL) and by proteolytic cleavage of caspase precursors and PARP-1. DNA fragmentation data (Fig. 2) showed that lipoplatin produced an at least 3-fold higher fraction of TUNEL-positive, MLH1-proficient cells than TUNEL-positive, MLH1-deficient cells. This indicates that the susceptibility to apoptosis is higher in cells expressing MLH1 protein and is hence dependent on the presence of MLH1 protein. Representative immunoblot data for the proteolytic cleavage of PARP-1 and the precursors for caspase-3 and -7 for a 24-h treatment with various concentrations of lipoplatin and captured 48 h after the addition of lipoplatin are presented (Fig. 3). The level of the PARP-1 precursor was substantially reduced as a function of lipoplatin concentration, but to a similar extent in MLH1-proficient and -deficient cells. The respective values for the reduction were 48 versus 52% (250 μmol/l) and 68 versus 75% (350 μmol/l). In addition, lipoplatin did not seem to produce a substantially larger decline in the level of the

Fig. 3



A typical data set for proteolytic cleavage of the caspase-3, caspase-7 and PARP-1 full-length precursors in MLH1-proficient HCT116 + ch3 cells and MLH1-deficient HCT116 + ch2 cells as a function of lipoplatin concentrations. Cells were treated with various concentrations of lipoplatin for 24 h, collected 48 h post-lipoplatin addition and prepared for immunoblot analysis. Proteins extracted from whole-cell lysates were separated by SDS–PAGE, and detected by the respective primary and secondary antibodies. The complexes were visualized by enhanced chemiluminescence. β-Actin is used as the sample loading control.

Fig. 4



Effect of lipoplatin treatment on Trypan blue inclusion in MLH1-proficient (HCT116 + ch3, black) and MLH1-deficient (HCT116 + ch2, white) cells. Cells were treated with various concentrations of lipoplatin for 1 (a) or 24 h (b), collected 72 h post-lipoplatin addition, and subjected to staining with Trypan blue dye. Columns: proportion of Trypan blue-positive cells relative to untreated controls, presented as the mean ± SD of three independent experiments. Values with $P < 0.05$ are indicated (*).

caspase-3 precursor in MLH1-proficient cells than in MLH1-deficient cells. Indeed, quantitative analysis revealed that the level of caspase-3 precursor dropped by 23% in MLH1-proficient cells and by 19% in MLH1-deficient cells (450 μmol/l, compared to the untreated control). Likewise, MLH1-proficient cells did not seem to be more susceptible to cleavage of the caspase-7 precursor than MLH1-deficient cells. The respective values for the caspase-7 precursor were 28 versus 33% for (350 μmol/l) and 81 versus 88% (450 μmol/l).

MLH1 deficiency and effect on necrosis

Trypan blue assessment showed that a 1-h treatment with lipoplatin up to a concentration of 800 μmol/l produced no more than 10% Trypan blue-positive cells, irrespective of the MLH1 status (Fig. 4a). A 24-h treatment produced a higher (ranging from 1.4- to 2.1-fold) proportion of Trypan blue-positive MLH-proficient than Trypan blue-positive MLH-deficient cells (Fig. 4b). An isotoxic effect was thus observed at higher lipoplatin concentrations for MLH1-deficient than for MLH1-proficient cells (IC_{50}: 540 versus 425 μmol/l, 1.3-fold difference).

Loss of MLH1 and G_2/M checkpoint activation

It was determined whether the observed resistance of MLH1-deficient cells to lipoplatin was paralleled by the failure to activate cell cycle checkpoints. Three data sets are presented. A 6-h treatment with 250 μmol/l lipoplatin (Fig. 5a) or 350 μmol/l lipoplatin (data not shown) as well as a 24-h treatment with 200 μmol/l lipoplatin (Fig. 5b) produced a prominent (3-fold) and sustained arrest at the G_2/M transition of the cell cycle in both the MLH1-proficient subline and the MLH1-deficient subline. Similar observations were made in two out of three experiments assaying a 24-h treatment with 150 μmol/l lipoplatin (data not shown). In the third, a decline by 20% in the fraction of cells arrested at the G_2/M transition following the antecedent increase was observed in MLH1-deficient cells, but not in MLH1-proficient cells (Fig. 5c). MLH1-deficient cells thus retain the ability to halt the cell cycle at the G_2/M transition.

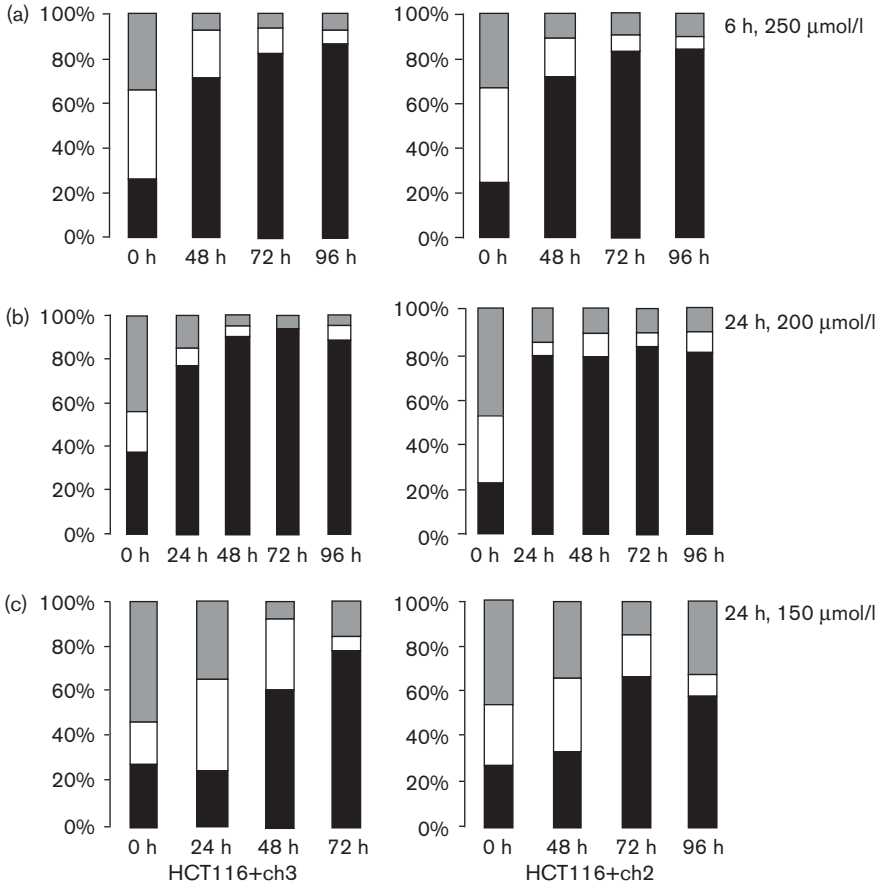
MLH1-deficiency and sensitivity to lipoxal

Lipoxal is liposome-encapsulated oxaliplatin. Oxaliplatin has been shown to exert its cytotoxic effect irrespective of the MMR status of cells [12]. Clonogenic data showed that MLH1-deficient HCT116 + ch2 cells were as sensitive to a 1- (Fig. 6a) or 24-h treatment (Fig. 6b) with lipoxal as MLH1-proficient HCT116 + ch3 cells. The respective IC_{50} values were 81 ± 11 versus 80 ± 14 μmol/l ($P = 0.87$) and 4.2 ± 0.5 versus 4.4 ± 0.5 μmol/l ($P = 0.57$). The cytostatic effect of lipoxal is thus not dependent on MLH1.

Discussion

We determined whether the efficacy of lipoplatin against tumor cells is dependent on the integrity of the function of MMR, a post-replicative DNA repair machinery that is implicated in initiating cytotoxic responses in the presence of damaged DNA. Here we demonstrate that (i) the sensitivity to lipoplatin, but not to lipoxal, was reduced in MLH1-deficient HCT116 tumor cells, (ii) the lipoplatin-resistance phenotype of MLH1-deficient cells was associated with reduced apoptosis, but not with the failure in activating the G_2/M checkpoint, and (iii) lipoplatin-induced, MLH1-dependent apoptosis did not correlate with processing of caspases and PARP-1. We may propose that MMR is a relevant contributor to the cytotoxic effect of lipoplatin and that caspase-independent pathways may be responsible for lipoplatin-induced, MMR-mediated apoptosis.

Fig. 5

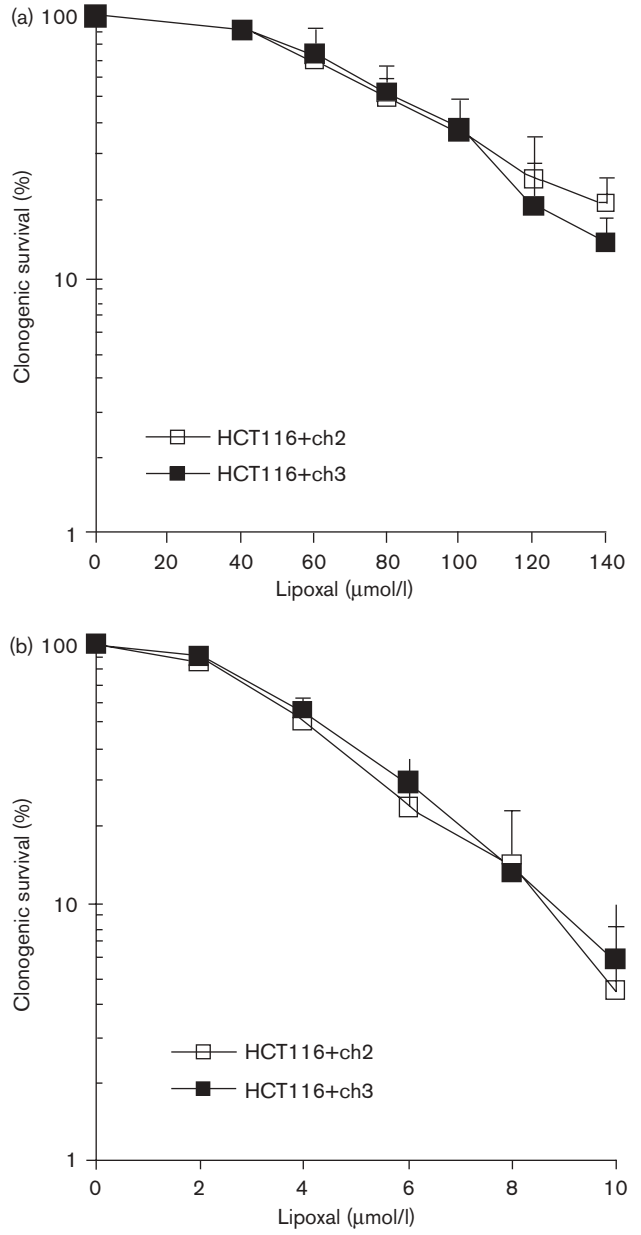


Effect of MLH1-deficiency on the ability of cells to attenuate progression of the cell cycle upon lipoplatin treatment. MLH1-proficient (HCT116 + ch3) cells and MLH1-deficient (HCT116 + ch2) cells were treated with various concentrations of lipoplatin for 6 (a) or 24 h (b and c), collected at the indicated time points post-lipoplatin addition, subjected to propidium iodide staining, and analyzed by flow cytometry. Three out of seven data sets are shown. Columns: distribution (expressed as the percentage of all cells, vertical axes) of MLH1-proficient HCT116 + ch3 cells and MLH1-deficient HCT116 + ch2 cells accumulated in the different phases of the cell cycle, presented as a function of time post-lipoplatin treatment: G_1/S, grey; S, white; G_2/M, black.

Our finding that the absence of the MMR protein MLH1 reduced the cytotoxic effect of lipoplatin indicates that MMR may be a relevant contributor to the efficacy of this drug against tumor cells. In contrast, this does not apply to lipoxal. These observations supplement previous studies reporting resistance to cisplatin and carboplatin, but not oxaliplatin, of cells deficient in either MLH1 or MSH2, another protein associated with MMR [12]. Likewise, the magnitude of lipoplatin resistance (2-fold) in MLH1-deficient cells compared with that reported for cisplatin and carboplatin [12]. However, at least in this in-vitro setting, the drug delivery from outside the cell to the nuclear target seemed to be less efficient for the encapsulated forms, since the isotoxic concentrations (e.g. IC_{50}) of lipoplatin and lipoxal were 10–20 times higher than those for the respective naked platinum complexes (Table 1). One explanation may be that naked cisplatin enters cells through the copper transporter channel [25], whereas liposome-encapsulated drugs enter cells through endocytosis [26]. Once within the cell naked cisplatin molecules are released from the liposome, become aquated and react with biomolecules including DNA to form several types of cisplatin–DNA adducts. Some of these are subject to recognition by MMR proteins, which subsequently elicits cell cycle checkpoint activation and apoptosis, probably via mechanisms similar to that proposed for alkylating agents [27,28]. As the nature of the initial formulation of the cisplatin complex seems irrelevant in this respect, lipoplatin may therefore, with regard to MMR, be proposed to act via mechanisms similar to those proposed for cisplatin.

The correlation of an apoptotic death of tumor cells after treatment of xenografts with lipoplatin with plasma pharmacokinetics and therapeutic efficacy in mouse xenografts with breast and prostate human tumors with

Fig. 6



Clonogenic survival of MLH1-proficient HCT116 + ch3 (■) and MLH1-deficient HCT116 + ch2 (□) cells in response to lipoxal treatment. Cells were treated with lipoxal for 1 (a) or 24 h (b), further incubated to allow colony formation, and stained with Giemsa on day 7 after lipoxal addition. Each data point represents the mean ± SD of four independent experiments.

an apoptotic death of tumor cells after treatment of xenografts with lipoplatin has previously been shown [6]. Our findings now indicate that lipoplatin-induced apoptosis (TUNEL DNA fragmentation) is dependent on the presence of MLH1 protein, which is also compatible with the proposed role for MMR in cisplatin-induced apoptosis [29]. Perhaps more noteworthy, MLH1-proficient cells did not show a greater decline in the level of the precursors for caspase-3, caspase-7 and PARP-1, indicating that MLH1-dependent apoptosis induced by lipoplatin is not paralleled by proteolytic processing of PARP-1 and caspases. This may mean that MMR-dependent apoptosis induced by lipoplatin could occur via caspase-independent mechanisms, being compatible with studies reporting that caspase-3 and caspase-7 are not indispensable for apoptosis [30], and that caspase-3 activation by cisplatin also occurs in MMR-deficient cells [31]. It has been reported that caspase-independent apoptosis can be performed by cathepsins and calpains [32–34], and that cisplatin-induced apoptosis in melanoma cells involves calpains rather than caspases [35]. The molecular mediators linking MMR with these proteases are unknown, but candidates may include molecules such as Bad, Bax and apoptosis-inducing factors [36].

Necrosis is another mechanism by which platinum drugs kill cells [37]. Our findings indicate that necrosis (Trypan blue inclusion) contributes to lipoplatin-induced cell death. Notably, MLH1 proficiency seemed to aggravate necrosis, suggesting that MMR contributes to necrosis. However, this apparent correlation may be due to the ability of Trypan blue to also stain TUNEL-positive (apoptotic) cells [38].

A previous study has shown that loss of MMR abrogates activation of the G_2/M checkpoint following cisplatin treatment [39], indicating that MMR is required for this checkpoint. Another study, however, has shown that loss of MMR is not sufficient for G_2/M transition checkpoint abrogation after cisplatin treatment [40]. Compatible with the latter study, our observations for lipoplatin indicate that MLH1-deficient cells retained the ability to attenuate cell cycle progression past the G_2/M transition, as lipoplatin treatment resulted in cell cycle progression attenuation at the G_2/M transition in both the MLH1-deficient cell line and the MLH1-proficient cell line. The extent of the G_2/M arrest was comparable and clearly sustained with time in both cell lines in five out of seven experiments (performed with various lipoplatin concentrations and treatment periods). A drop following the antecedent increase in the fraction of cells accumulated at this transition was observed for MLH1-deficient cells, but not for MLH1-proficient cells, in the two other experiments, but the absence of G_2/M checkpoint activation was never observed. This indicates that MLH1 is not required for this checkpoint, suggesting that its activation by lipoplatin is MLH1 independent.

The involvement of MMR in DNA damage recognition, correction and signaling seems to play an important role in the prevention of carcinogenesis and in the responses of cancers to chemotherapeutic agents. The MMR defect is found in several cancers, both familiar and sporadic, and MMR-deficient cells are 100-fold resistant to killing by

Table 1 Comparison of IC_{50} values (means ± SD) for lipoplatin versus cisplatin and lipoxal versus oxaliplatin in HCT116 + ch2 and HCT116 + ch3 sublines

	Treatment			
	24-h		1-h	
	HCT116 + ch2	HCT116 + ch3	HCT116 + ch2	HCT116 + ch3
Lipoplatin (μmol/l)	70 ± 11	38 ± 8	556 ± 66	359 ± 39
Resistance[a]	2.3-fold[d]		1.6-fold[d]	
Cisplatin (μmol/l)	2.8 ± 0.6[b]	1.7 ± 0.1[b]	23 ± 4[c]	11 ± 4[c]
Resistance[a]	1.6-fold		2.1-fold	
Lipoxal (μmol/l)	4.2 ± 0.5	4.4 ± 0.5	81 ± 11	80 ± 14
Oxaliplatin (μmol/l)	1.3 ± 0.2[b]	1.4 ± 0.3[b]	14 ± 4[c]	16 ± 4[c]

[a]Resistance, expressed as the ratio of IC_{50} values (HCT116 + ch2 versus HCT116 + ch3).
[b]Unpublished data.
[c]From Fink *et al.* [12].
[d]$P < 0.01$.

methylating agents of the S(N)1 type than cells with functional MMR. In the case of cisplatin–DNA adducts, the sensitivity difference was lower, typically 2- to 3-fold, but was observed in all matched MMR-proficient and -deficient cell pairs (reviewed in [18]). Loss of MMR is a significant mechanism of acquired drug resistance in *dam* bacteria, but in human cells the contribution of defective MMR to acquired resistance to cisplatin is less important [41]. In contrast, the diaminocyclohexane adducts of oxaliplatin are not substrates for the MMR system [12,42]. Accordingly, the absence of functional MMR negatively affects the efficacy of lipoplatin, but does not affect the efficacy of lipoxal. Thus, the observed MMR independence of the cytostatic effect of lipoxal suggests that lipoxal might be preferred to lipoplatin in the treatment of cancers deficient in MMR.

In conclusion, we showed that loss of MMR function was associated with a lipoplatin resistance phenotype in tumor cells. This may identify MMR function as a relevant mediator for the cytotoxicity of lipoplatin against tumor cells, which seems to occur via caspase-independent apoptotic pathways.

Acknowledgments

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