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Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines

S. Arnould[a], I. Hennebelle[a], P. Canal[a], R. Bugat[a,b], S. Guichard[a,b,*]

[a]*Groupe de Pharmacologie clinique et expérimentale (EA 3035), Institut Claudius Regaud, 20-24, rue du pont St Pierre, 31052 Toulouse cedex, France*

[b]*Université Paul Sabatier, 118, route de Narbonne, 31062 Toulouse cedex, France*

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Abstract

Oxaliplatin (L-OHP) is a new platinum analogue that has shown antitumour activity against colon cancer both *in vitro* and *in vivo* and is now used in the chemotherapeutic treatment of metastatic colon and rectal cancer. L-OHP like cisplatin (CDDP), is detoxified by glutathione (GSH)-related enzymes and forms platinum (Pt)–DNA adducts lesions that are repaired by the nucleotide excision repair system (NER). We investigated the cytotoxicity and the pharmacology of L-OHP and CDDP on a panel of six colon cell lines *in vitro*. We showed that GSH and glutathione S-transferase (GST) activity were not correlated to oxaliplatin cytotoxicity. Pt–DNA adducts formation and repair were correlated with CDDP, but not with L-OHP cytotoxicity. The determination of *ERCC1* and *XPA* expression, two enzymes of the NER pathway, by reverse transcriptase-polymerase chain reaction (RT-PCR), demonstrated that *ERCC1* expression was predictive of L-OHP sensitivity ($r^2 = 0.67$, $P = 0.02$) and *XPA* level after oxaliplatin exposure was also correlated to L-OHP IC_{50} ($r^2 = 0.5$; $P = 0.04$). The knowledge of such correlations could help predict the sensitivity of patients with colon cancer to L-OHP.

Keywords: Oxaliplatin; Cisplatin; Colon cancer; Glutathione; DNA adducts; Nucleotide excision repair

1. Introduction

Oxaliplatin (L-OHP) is a new platinum (Pt) analogue that differs from cisplatin (CDDP) by the presence of a diaminocyclohexane ligand (DACH) in its chemical structure. L-OHP and CDDP act theoretically by the same mechanism of action, i.e. alkylation of DNA: L-OHP is monoaquated in the cells and reacts with the (N)7 of guanines on the DNA forming monoadducts that are converted into diadducts over time. L-OHP, like CDDP, is inactivated by reaction with glutathione catalysed by the glutathione-S-transferase (GST) enzyme. L-OHP and CDDP adducts are both repaired by the nucleotide excision repair (NER) system from which two enzymes, XPA and ERCC1 have been identified as being essential for the repair process [1,2]. Despite these similarities, several studies have shown differences between the two compounds. The National Cancer Institute (NCI) in screening studies showed a clustering of the DACH Pt compounds that is very different from the other platinum compounds, making the DACH compounds a new family of Pt derivatives [3]. Moreover, CDDP-resistant cell lines were sensitive to L-OHP [3,4]. Finally, L–OHP was active in colon and rectal cancers, while CDDP was not [5]. Although L-OHP forms Pt–DNA adducts, these adducts are bulkier and induce a greater deformation of the DNA structure than CDDP–DNA adducts [6–8]. Consequently, L-OHP is more effective in inhibiting DNA synthesis [8,9]. Moreover, while CDDP DNA adducts are recognised by the mismatch repair system, the distortion of DNA caused by L-OHP adducts prevent binding on the mismatch repair complex [6,10,11]. The clinical activity of L–OHP remains low, with 17% of clinical responses in phase II studies [5], and was improved by combining it with 5-fluorouracil (5-FU) [12]. The pharmacoogy of L-OHP remains partially

* Corresponding author at present address: Cancer Research UK Building, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XR, UK. Tel.: +44-131-777-3556; fax: +44-131-777-3520.
E-mail address: sylvie.guichard@cancer.org.uk (S. Guichard).

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understood, especially the cellular determinants that control the cytotoxic effect of this compound. A better knowledge of these determinants could help explain the very different spectrum of activity of L-OHP and CDDP.

We investigated further the mechanisms of action of L-OHP and CDDP in a panel of six colon cancer cell lines and compared the results for the two compounds.

2. Materials and methods

2.1. Cell culture

HT-29, HCT-8, SW620, HCT-116, COLO205 and Lovo cell lines were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA). Cells were grown in monolayers in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% (v/v) fetal calf serum (FCS) and 1% (v/v) L-glutamine at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were split once a week using Trypsin/ethylene diamine tetra acetic acid (EDTA) (0.25%/0.02% (v/v)). Doubling times were 24 h for HCT-8 and HCT-116, 26 h for COLO 205, and 28 h for HT-29, LoVo and SW620.

2.2. Cytotoxicity assay

Drug concentrations that inhibit 50% of cell growth (IC_{50}) were determined using the sulforhodamine B technique [13]. Cells were plated on day 1 in 96-well plates. The cell density was 1500 cells/well for HCT-8 and HCT-116, 2200 cells/well for HT-29 and LoVo, and 4500 cells/well for SW620 and COLO205 in a volume of 150 μl/well. The cell density was chosen to have a cell growth latency of 24 h and an optical density at the end of the experiment that was greater than 1.5 in the control wells. In each plate, one column contained control cells, and nine columns increasing concentrations of the drugs. For each column, six wells were used. Cells were treated on day 2 with L-OHP for 1 h (final concentration range: 0.5–250 μM) or 24 h (range: 0.025–10 μM) or with CDDP for 1 h (final concentration range: 1–500 μM) or 24 h (final concentration range: 0.05–25 μM) in a volume of 50 μl. After drug exposure, the medium was replaced with 200 μl of drug-free medium and cells were grown for 72 h after the end of drug exposure. The cells were then precipitated with 50 μl of ice-cold 50% (w/v) trichloracetic acid for 60 min at 4 °C, rinsed six times with water and air-dried. Fixed cells were stained with 50 μl of 0.4% (w/v) of sulforhodamine B solution in 0.1% (v/v) acetic acid, rinsed with 0.1% acetic acid solution and air-dried. Sulforhodamine B was re-dissolved with 150 μl of 10 mM Tris buffer, pH 10.5. Optical densities (OD) were measured at 540 nm with a Multiskan multisoft apparatus (Labsystems, Les Ulis, France). Growth-inhibition curves were plotted as a percentage of control cells and the IC_{50}s were determined by an interpolated graph. The results are based on six independent experiments.

2.3. Preparation of cytosolic protein

Exponentially growing cells (3×10^6 in a Petri dish) were harvested in phosphate-buffered saline (PBS) containing 50 μg/ml leupeptin, centrifuged at 1200*g* for 10 min. The pellets were frozen at −80 °C until analysis. Pellets were then resuspended in 10 mM Tris, 1.5 mM EDTA buffer containing 50 μg/ml leupeptin, sonicated, and centrifuged at 45 000*g* for 30 min. Cytosolic protein content was determined by the Bradford method [14].

2.4. Determination of intracellular glutathione (GSH) levels

GSH levels were determined according to Akerboom and Sies [15]. The reduction of 5,5′dithiobis(2nitrodithiobis(2nitrobenzoic acid) (DTNB) by GSH was monitored at 414 nm on a Multiskan multisoft apparatus (Labsystems, Les Ulis, France) in a kinetic mode. The reaction was started by mixing the samples (130 μg/ml) or GSH standards (1.25–25 nmol/ml oxidised glutathione) in a volume of 25 μl with 250 μl of reaction mixture (0.2 ng/ml of Nicotinamide Adenine Dinucleotide Phosphate, Reduced (NADPH), 0.03 mg/ml DTNB, 0.2 U/ml GSH reductase in 100 mM potassium phosphate buffer/1 mM EDTA pH 7). OD measures were taken every 10 s for 2 min. Results were expressed as nmol total GSH/mg protein and are the mean of three independent experiments.

2.5. Determination of glutathione-8-transferase (GST) activity

GST activity was determined according to Habig [16]. The conjugation of 1-chloro-2,4dinitrobenzene (CDNB) to GSH by GSH synthetase was monitored at 340 nm. The difference of absorbance during the first 3 min was a linear function of the GST activity. Cytosols (25 μl) were incubated with reduced GSH for 2 min. The reaction was started by adding CDNB (2 mM final concentration). Ten OD measurements were taken every 10 s. Results were expressed as U of GST/mg protein. Three independent experiments were performed.

2.6. L-OHP and CDDP DNA adduct formation and repair

Exponentially growing cells were treated with 40 μM oxaliplatin or 50 μM CDDP for 1 h. At the end of incubation, cells were rinsed twice with cold PBS and either frozen at −20 °C or placed in drug-free medium

for 1 and 24 h. For 24-h time points, cells were incubated with 2.5 μCi [^{3}H] thymidine 24 h prior to L-OHP exposure in order to correct the DNA adduct concentrations from replication.

Frozen cells were scraped with 1 ml lysis buffer (0.3 M NaCl, 5 mM EDTA, 0.5% (w/v) sodium dodecyl sulphate (SDS), 50 μg/ml proteinase K). After incubation overnight at 37 °C, DNA was extracted successively with Tris-saturated phenol and phenol/chloroform. DNA was precipitated with 10 M ammonium acetate and 95% (v/v) ethanol and washed with 70% (v/v) ethanol. DNA was re-dissolved in water and stored at −20 °C until analysis. The DNA concentration was determined at 260 nm and was then digested with S1 nuclease overnight at 37 °C.

Pt content was determined by atomic absorption spectroscopy (AAS) on an Aanalyst 600 (Perkin–Elmer, Courtaboeuf France). Temperature steps were: 120 °C for 20 s, 130 °C for 50 s, 350 °C for 20 s, 550 °C for 20 s, 1400 °C for 20 s and 2350°C for 8 s (reading at 266 nm). Pt concentrations in samples were calculated from a standard curve (5.25–262.5 pg Pt/μg). Pt–DNA adducts level were expressed as pg Pt/μg DNA. Concentrations determined 24 h after the end of incubation were corrected by the ratio of thymidine incorporation between the start of the incubation and the 24-h time point. Results are mean±standard deviation (S.D.) of six independent experiments.

2.7. Determination of XPAC and ERCC1 expression by RT-PCR

Total RNA was extracted using Trizol solution (Life Technologies, Cergy-Pontoise, France). Two micrograms of RNA were reversed transcribed using Ready-to-go You prime first-strand kit (Amersham Pharmacia Biotech, Inc., Piscataway NJ, USA). The following primers were used: *XPA* primers (XPAS:$^{5'}$GCATGG CTAATGTAAAAGCA$^{3'}$;XPA AS:$^{5'}$TCCTGTGGACT TCCTTTGC$^{3'}$), *ERCC1* primers (ERCC1S:$^{5'}$GAGCT GGCTAAGATGTGTAT$^{3'}$; ERCC1 AS: $^{5'}$AGGCCA GATCTTCTCTTGAT$^{3'}$), and *β-actin* primers (BAS:$^{5'}$ ATCTGGCACCACACCTTCTACAATGAGCTGCG$^{3'}$) and (BA AS: $^{5'}$CGTCATACTCCTGCTTGCTGATCC ACATCTGC$^{3'}$).

The 100-μl reaction mixture contained 10 mM Tris pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% (v/v) Triton X100, 0.2 mg/ml bovine serum albumin (BSA), 50 μM of each deoxynucleotide triphosphate (dNTP), 0.5 U Taq polymerase and either 20 pmol of *XPA* primers, 30 pmol of *ERCC1* primers, or 5 pmol of *β-actin* primers. Amplification was: denaturation 1 min at 94 °C, hybridisation 1 min at 60 °C, and extension 1 min 30 s at 72 °C, for 35 cycles. Reaction products were electrophoretically separated in a 2% agarose gel in TBE buffer and visualised after ethidium bromide staining. Polymerase chain reaction (PCR) products were quantitated using Image Quant software.

Results are expressed as the ratio of intensity of the bands from *ERCC1*, *XPA* and *β-actin*. Results are mean ±S.D. of four independent experiments.

3. Results

3.1. Cytotoxicity of 1- and 24-h exposure to cisplatin and oxaliplatin

Cytotoxic effects of both CDDP and L-OHP were determined using 1- and 24-h exposures in a panel of colon cell lines: HT-29, SW620, HCT-8, HCT-116, COLO205 and LoVo (Table 1). Fig. 1 presents the ratio of IC_{50}s between CDDP and L-OHP in this panel. Overall, the colon cell lines selected were more sensitive to L-OHP than to CDDP except HCT-116 which exhibited a higher sensitivity to CDDP than to L-OHP (18.6 μM versus 38.2 μM).

The time-dependence index (TDI) was determined as the ratio between the IC_{50} obtained for the 1-h exposure and the IC_{50} for the 24-h exposure. TDIs ranged from 8.5 to 24.2 for CDDP and 28.5 to 90.5 for L-OHP. Due to the high TDI observed for L-OHP, the correlations

Table 1
IC_{50} values of L-OHP and CDDP after 1- and 24-h exposure in a panel of six colon cell lines

Cell line	CDDP (μM)			L-OHP (μM)		
	1-h exposure	24-h exposure	TDI[a]	1-h exposure	24-h exposure	TDI
LoVo	28.3±2.4	2.2±0.3	11.8	27.6±11.1	0.3±0.1	90.5
COLO205	50.7±3.2	2.4±0.3	23.3	18.4±3.6	0.3±0.2	71.9
SW620	25.3±9.9	1.9±0.5	12.8	11.9±6.0	0.4±0.04	38.9
HT-29	62.6±5.9	2.4±0.4	24.2	23.8±8.1	0.9±0.6	28.5
HCT-116	18.6±3.8	2.5±1.4	8.5	38.2±8.2	0.7±0.2	54.3
HCT-8	77.2±9.4	3.9±1.3	21.9	44.4±3.9	0.8±0.3	61.5

L-OHP, oxaliplatin; CDDP, cisplatin.
[a] TDI (time-dependence index), the ratio between IC_{50} after 1-h exposure and IC_{50} after 24-h exposure.

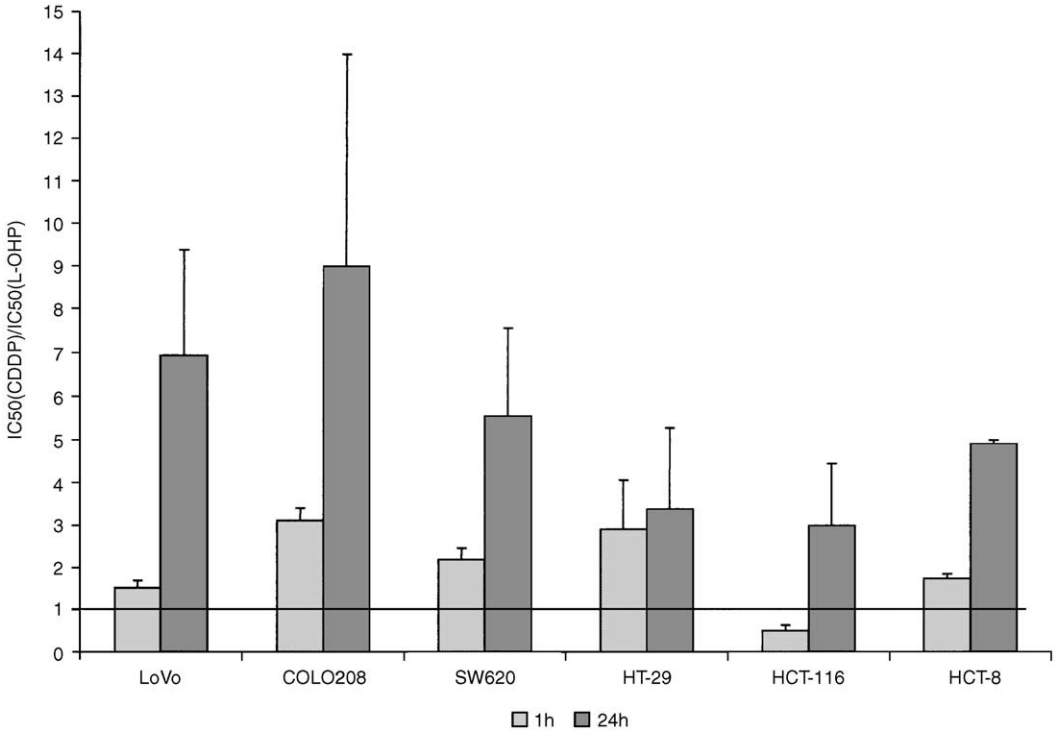

Fig. 1. Ratios between IC_{50} values for cisplatin (CDDP) and oxaliplatin (L-OHP) using 1- and 24-h exposure. Cytotoxicity was evaluated by sulforhodamine B technique in 96-well plates. Cells were plated on day 0, treated on day 1, and cytotoxicity measured three doubling times after drug exposure. Results are the mean of six independent experiments.

established with cellular parameters were done with the 24-h exposure's IC_{50}.

3.2. Concentration of intracellular GSH and GST

Fig. 2 shows the intracellular GSH concentration (Fig. 2a) and the GST activity (Fig. 2b) in HT-29, SW620, HCT-8, HCT-116, COLO205 and LoVo cell lines. GSH content varied widely in our panel of colon cell lines from 1.3 nmol/mg protein in the HCT-8 cells to 4.1 nmol/mg protein in the COLO205 cells. The GST activity was the lowest (96.9 U/mg protein) in HCT-116 cells. COLO205 cells exhibit the highest activity (297.6 U/mg protein). There was no relationship between either GSH content or GST activity and CDDP or L-OHP cytotoxicity.

3.3. Pt–DNA adducts after CDDP and L-OHP exposure

Table 2 presents the formation and removal of Pt–DNA adducts after 40 μM oxaliplatin and 50 μM CDDP treatment in our panel of six colon cancer cell lines (mean IC_{50} of each drug in our panel). CDDP–DNA adducts ranged from 103.3 pg Pt/μg DNA in HCT-8 cells to 209.9 pg/μg DNA in HT-29 cells. The repair of these adducts was rapid with only 5–12 and 1–7% of the initial level remaining 1 and 24 h after exposure, respectively. The level of DNA adducts formed at the end of exposure was not correlated with the cytotoxicity of CDDP ($r^2 = 0.09$). However, the level of Pt–DNA adducts 24 h after exposure was related to CDDP IC_{50} (1-h exposure), albeit not statistically significant ($r^2 = 0.37$, $P = 0.2$): the higher the level of Pt adducts, the lower the CDDP IC_{50}.

L-OHP–DNA adducts levels ranged from 22.2 pg/μg DNA in HT-29 cells to 45.3 pg Pt/μg DNA in COLO205 cells. The removal of the adducts was slower with 11–43% of the initial level 1 h after exposure and 6–24% remaining after 24 h. A linear relationship existed between the initial and the residual levels of Pt–DNA adducts after L-OHP exposure ($r^2 = 0.42$, $P = 0.08$). However, neither the level of DNA adducts at the end of exposure or the residual level 24 h after exposure were significantly correlated to L-OHP IC_{50} ($r^2 = 0.21$ and 0.24, respectively).

3.4. Determination of XPA and ERCC1 mRNA levels by RT-PCR

The level of expression of *XPA* and *ERCC1*, two proteins of the NER process was determined by RT-PCR after treatment of the cells with either 40 μM L-OHP or 50 μM CDDP for 1 h. The results are expressed as a ratio of the expression between the gene of interest (*XPA* or *ERCC1*) and the *β-actin* house-

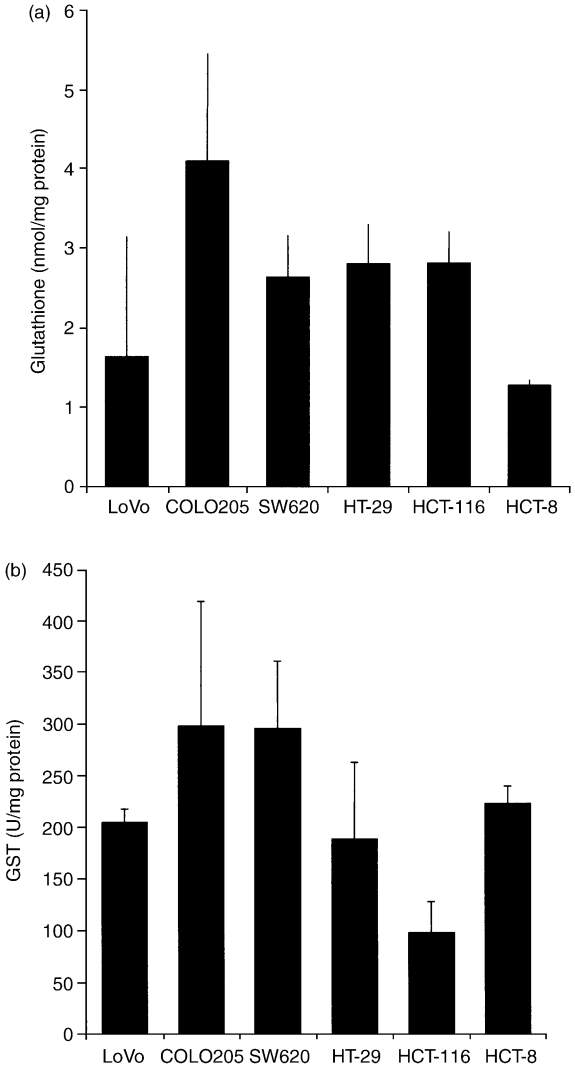

Fig. 2. (a) Glutathione (GSH) (nmol/mg protein) levels in our panel of six colon cell lines. GSH levels were determined by 5,5′dithiobis(2nitrobenzoic acid) (DTNB)'s conversion. (b) Glutathione-S-transferase (GST) activity (U/mg protein) in our panel of six colon cell lines evaluated by the conjugation of 1-chloro-2,4dinitrobenzene (CDNB) to glutathione. Results in each case are the mean of three independent experiments.

keeping gene. The basal level of *ERCC1* was correlated to the 24-h residual amount of CDDP–DNA adducts: the lower the initial level of *ERCC1*, the higher the residual amount of Pt–DNA adducts ($r^2 = 0.5$; $P = 0.06$). Moreover, *ERCC1* basal level was related to CDDP IC_{50}: the higher *ERCC1* level, the higher the CDDP IC_{50}. Conversely to CDDP, *ERCC1* basal level was not correlated to residual level of L-OHP–DNA adducts ($r^2 = 0.15$). However, a correlation between *ERCC1* basal level and L-OHP IC_{50} was observed ($r^2 = 0.67$; $P = 0.02$) (Fig. 3). No correlation was seen between *XPA* expression and CDDP adducts or cytotoxicity. However, the basal level of *XPA* seems to be linked with L-OHP residual adducts level. Moreover, L-OHP

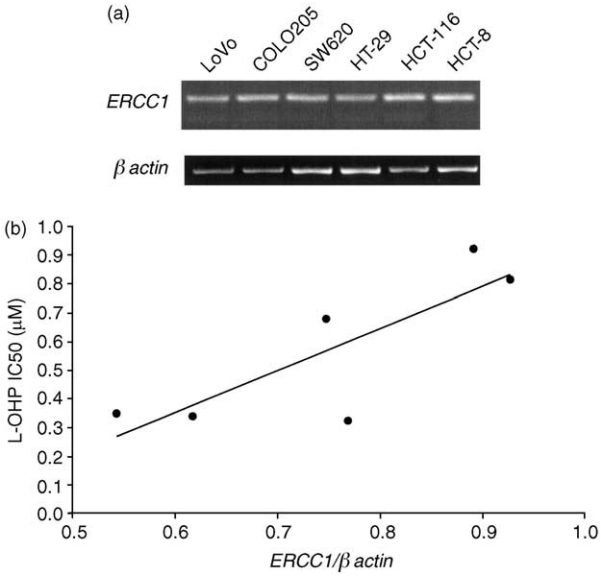

Fig. 3. (a) *ERCC1* and *β-actin* expression determined by reverse transcriptase-polymerase chain reaction (RT-PCR) on a panel of six colon cell lines. Electrophoresis of PCR products was performed on 2% agarose gel in TBE buffer. (b) Correlation between *ERCC1* expression and oxaliplatin (L-OHP) cytotoxicity. *ERCC1* expression was evaluated by RT-PCR on the six colon cell lines and correlated with the cytotoxic effect of L-OHP determined by sulforhodamine B technique. Results are the mean of four independent experiments.

Table 2
Pt–DNA adducts levels determined in six colon cell lines after 1-h exposure to 50 μM CDDP and 40 μM L-OHP.

	CDDP			L-OHP		
pg Pt/μg DNA	End of exposure	After 1 h	After 24 h	End of exposure	After 1 h	After 24 h
LoVo	153.3±62.9	18.5±8.5	10.7±7	23.9±15.4	5.8±6.6	5.8±8.7
COLO205	208.1±56.1	14.3±8.0	5.1±1.6	45.3±46.7	5.1±2.5	2.7±1.6
SW620	169.7±18.8	10.9±6.9	4.6±0.3	40.8±33.7	9.9±10.1	3.0±4.7
HT-29	209.9±45.5	14.7±0.1	3.0±1.2	22.2±11.6	9.1±5.7	3.2±4.1
HCT-116	188.1±19.5	9.6±8.1	7.3±2.5	23.7±16	10.3±9.5	4.4±4.2
HCT-8	103.3±3.4	11.2±1.6	4.9±2.2	33.4±19	5.2±6.1	4.0±3.5

Pt, platinum; CDDP, cisplatin; L-OHP, oxaliplatin.
The Pt–DNA adducts were measured at the end of the 1-h exposure, and then 1 and 24 h after the removal of the drug.

induced an increase of *XPA* mRNA expression, an increase that was correlated with the cytotoxic effect ($r^2 = 0.55$; $P = 0.04$).

4. Discussion

L-OHP is a new Pt compound that showed an antitumour effect in colorectal cancer, where other Pt compounds have failed to demonstrate activity. 5-FU improves the clinical activity of L-OHP and this combination is now used as first-line treatment for metastatic colon or rectal cancer in France with a 42% clinical response [17]. These results, albeit very attractive, mask a very limited knowledge of the pharmacology of L-OHP. We investigated the cytotoxic effect and the pharmacology of L-OHP compared with CDDP on a panel of colon cell lines that exhibited a large variation of L-OHP and CDDP sensitivity, as well as mismatch repair defect and *P53* mutation. Cytotoxicity of L-OHP and CDDP was evaluated at two different time exposures: 1 and 24 h. The cell lines from our panel were more sensitive to L-OHP than to CDDP with the exception of the HCT-116 cell line. Rixe and colleagues already reported this particular feature of the HCT-116 cells [3]. The TDI calculated from the IC_{50} values was greater for L-OHP than for CDDP. The slow conversion of L-OHP–DNA adducts compared with CDDP, as shown by Saris and colleagues [18] could explain the difference in TDI between CDDP and L-OHP.

Since several studies have suggested GSH and GSH-related enzymes are involved in the sensitivity of cells to Pt compounds [19], GSH level and GST activity were determined in our panel of colon cell lines. No correlation could be established between either GSH or GST and cytotoxicity to CDDP and L-OHP. This result is consistent with the study from Pendyala and colleagues who could not demonstrate similar correlations with L-OHP in a panel of cell lines from different origins [20]. Although it is clear that detoxification can be a resistance factor during a selection process, the overall sensitivity of a cell line is multifactorial. Therefore, the contribution of detoxification to resistance is difficult to establish.

CDDP and L-OHP act by alkylating DNA and the level of Pt–DNA adducts is thought to be a main factor in Pt-compound cytotoxicity. We observed a higher level of DNA adducts after CDDP than after L-OHP exposure. On average, there was 5-fold less L-OHP–DNA adducts than CDDP–DNA adducts for the same cytotoxic effect. Several studies described the low reactivity of L-OHP compared with CDDP with DNA [18,21,22] which contrasted with a greater cytotoxic effect of L-OHP. In particular, the DACH carrier ligand of L-OHP slowed the rate of monoadduct to diadduct conversion [7]. However, *in vitro*, the rate of repair of the intrastrand adducts seemed to be identical for CDDP and L-OHP [23]. The presence of L-OHP adducts in highly transcribed regions of the genome could be a reason for this higher cytotoxicity despite a lower DNA platination. Other mechanisms of cytotoxicity have been suggested for L-OHP, like DNA–protein crosslinks [21]. Other authors suspected that L-OHP–DNA adducts were not recognised by the DNA repair systems in the same way that CDDP–DNA adducts were. This has been demonstrated for the mismatch repair system [6,10,11]. Polymerases are usually stopped by Pt–DNA adducts, but polymerase beta has the ability to bypass the lesions and to operate a translesion synthesis. Moreover, Pt–DNA adducts are thought to be recognised by high-mobility group protein I (HMG1) which further recruits DNA repair proteins. Vaisman and colleagues demonstrated that polymerase beta was able to bypass L-OHP–DNA adducts and that the ability of the HMG1 protein to block translesion synthesis operated by polymerase beta was 2.5-fold lower for L-OHP than for CDDP–DNA adducts [22]. So, L-OHP adducts are, in themselves, better bypassed by polymerase beta, and this phenomenon is amplified when HMG1 proteins are present on the adducts. This is consistent with our data showing that while the residual level of Pt–DNA adducts is related to CDDP cytotoxicity, this is not the case for L-OHP. This is consistent with the results of Schmidt and colleagues [24] in HCT-8 and A2780 cells showing that there was no direct correlation between the formation/repair of Pt–DNA adducts and the cytotoxicity of L-OHP. This confirmed that for oxaliplatin, the level of DNA adducts is not the major factor for the cytotoxicity but it is the way the cells either deal with the mutations induced by translesion synthesis or repair the lesions induced by oxaliplatin, that is more important.

In order to evaluate the importance of the nucleotide excision repair (NER) process in the sensitivity to L-OHP and CDDP, the expressions of *XPA* and *ERCC1* were determined by RT-PCR. XPA is involved in the very early steps of DNA damage recognition, associated with Replication Protein A. It plays a major role in NER since it was demonstrated that mutations on *XPA* that prevent the interaction between *XPA* and *ERCC1* abolished DNA repair [25]. Moreover, the increase in expression of *XPA* in testicular germ cell tumours that presented initially low levels of XPA can restore their ability to remove CDDP–DNA adducts [26]. ERCC1 is an endonuclease that was shown to be overexpressed in CDDP-resistant cells, both *in vitro* and in tumour samples [27]. In our experience, the initial level of expression of *ERCC1* was correlated to both the residual level of CDDP–DNA adducts and CDDP cytotoxicity: a high level of *ERCC1* induced a greater removal of CDDP DNA adducts and, consequently, a lower cytotoxic effect. The initial level of *ERCC1* is predictive of L-OHP cytotoxicity, although it is not

related to L-OHP–DNA adducts. So, while the level of Pt–DNA adducts and the *ERCC1* expression seemed to be linked for CDDP activity, there was a decoupling between the adduct level and *ERCC1* expression for L-OHP. Moreover, we observed a correlation between *XPA* expression after L-OHP exposure and cytotoxicity. When cells are able to keep *XPA* at a low level or even to downregulate *XPA*, the residual level of Pt DNA adducts increase inducing a higher cytotoxic effect. It is unclear if the increase in the mutation rate due to translesion synthesis could lead to L-OHP resistance, but the HCT-8 cells, which in our experience are resistant to L-OHP, were reported to have a high level of polymerase β [28].

L-OHP and CDDP both act by alkylating DNA, but the two drugs can be distinguished by the way in which the cells deal with the adducts formed and are able to either tolerate them or to repair them. NER plays an essential role in L-OHP sensitivity, through XPA and ERCC 1. We showed that the *ERCC1* level is predictive of L-OHP cytotoxicity. *XPA* level after L-OHP exposure is also related to oxaliplatin activity. The clinical value of such correlations in the prediction of L-OHP sensitivity could be tested in cancer patients by determining *ERCC1* and *XPA* expression in tumour samples and comparing them with clinical outcome. Shirota and colleagues have recently demonstrated the predictive value of *ERCC1* expression in tumour samples from patients with colorectal cancer treated with L-OHP and 5-FU [29]. Further experiments will be necessary to evaluate the exact role of polymerase β in L-OHP cytotoxicity in colon cancer.

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