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Acknowledgements—This work was supported by grants from the Helen Buchanan and Stanley Joseph Seeger Endowment for General Surgery and the Elisa U. Pardee Foundation.

Eur J Cancer, Vol. 27, No. 8, pp. 1039–1045, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Comparative Study of Two Human Melanoma Cell Lines with Different Sensitivities to Mustine and Cisplatin

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The cytotoxic effects of the bifunctional DNA-reactive drugs cisplatin, mustine and melphalan were studied in two human melanoma cell lines, RPMI 8322 and A 375. A 375 cells were 3.0 times more sensitive to cisplatin and 1.5 times more sensitive to mustine than RPMI 8322 cells. In contrast, A 375 cells were less sensitive to melphalan than RPMI 8322 cells. The increased sensitivity of A 375 cells was parallelled by an increased induction of DNA interstrand crosslinks following exposure to cisplatin and mustine. After cisplatin exposure A 375 cells also showed higher levels of platinum-DNA intrastrand adducts than RPMI 8322 cells. The increased effect of cisplatin in A 375 cells was not due to an increased drug accumulation in these cells. The higher sensitivity of A 375 cells to cisplatin may be related to lower intranuclear levels of glutathione, compared to RPMI 8322 cell nuclei, while the sensitivity to mustine may depend on lower overall levels of glutathione than in RPMI 8322 cells.
Eur J Cancer, Vol. 27, No. 8, pp. 1039–1045, 1991.

INTRODUCTION

CIS-DIAMMINEDICHLOROPLATINUM(II) (cisplatin) is an important chemotherapeutic drug, which has shown clinical activity in several different tumour types [1]. Malignant melanoma, however, is generally resistant to cisplatin therapy. Only 10% of patients with disseminated melanoma obtain objective tumour responses following treatment with cisplatin as a single agent [2].

There is considerable evidence that the cytotoxic effects of cisplatin are caused by reactions with DNA [3]. It has been demonstrated that cisplatin induces DNA intrastrand crosslinks, DNA interstrand crosslinks and DNA–protein crosslinks [4, 5]. It is not known which of these lesions are responsible for the cytotoxic effect of cisplatin.

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Revised 15 Apr. 1991; accepted 17 Apr. 1991.

During recent years several studies on the mechanisms of resistance of tumour cells to cisplatin have been performed. Most investigators have compared sensitive parent cell lines to daughter cell lines which had been made resistant *in vitro* by exposure to high levels of cisplatin. Several mechanisms contributing to such *in vitro*-induced resistance have been described. These include: reduced intracellular accumulation of the drug [6–12]; increased levels of glutathione [7, 13–16] or increased glutathione transferase (GST) activity [6], which may play a role in inactivating cisplatin; reduced induction of DNA lesions by cisplatin [6, 17]; and increased DNA repair [8, 14, 18]. In addition, increased levels of metallothionein have been demonstrated in cell lines with increased resistance to cadmium, which were crossresistant to cisplatin [19]. Binding of cisplatin to metallothionein was also demonstrated in this study. While these studies have demonstrated several biochemical mechanisms for induced cellular resistance to cisplatin, it remains to be established whether these are important for the intrinsic resistance of human tumours such as malignant melanoma.

Fewer studies have been performed on human cells with inherent differences in cisplatin sensitivity [20, 25]. Such studies may be more relevant for demonstrating which mechanisms

determine the response of tumours to cisplatin therapy in clinical practice. Early investigations of human normal and tumour cell lines showed a correlation between sensitivity to cisplatin and the induction of DNA interstrand crosslinks [20, 21]. More recently, Bedford *et al.* compared two human bladder carcinoma cell lines with a 2-fold difference in sensitivity to cisplatin [22]. The more resistant cells were found to have decreased binding of platinum to DNA and lower levels of DNA interstrand crosslinks despite a similar uptake of drug in the two cell lines. This might be due to increased inactivation of cisplatin since the resistant cell line had increased levels of glutathione and increased GST and glutathione reductase activities. The more resistant of the two bladder cancer cell lines has also been compared to two cisplatin-sensitive testicular tumour cell lines [23]. Both testicular tumour cell lines showed increased accumulation of cisplatin compared with the bladder carcinoma cells. In the more sensitive of the two cell lines, a deficiency in removal of both DNA intrastrand and DNA interstrand crosslinks was demonstrated. In an investigation by Pera *et al.*, three cisplatin-sensitive embryonal carcinoma cell lines were found to be hypersensitive to the presence of cisplatin–DNA adducts compared to normal fibroblasts, but the reason for this sensitivity was not explored further [24].

We have previously shown that the human melanoma cell line RPMI 8322 is resistant to cisplatin compared to phytohaemagglutinin-stimulated human lymphocytes [25]. In the present study we have compared the RPMI 8322 cell line to another human melanoma cell line, A 375, which is more sensitive to cisplatin. In order to investigate some of the mechanisms responsible for the difference in cisplatin sensitivity, several comparisons between the two cell lines have been performed. Thus, we have examined the sensitivities of the two cell lines to two other bifunctional DNA reactive drugs, melphalan and mustine. Differences in drug sensitivity have been related to DNA interstrand crosslinking following exposure to cisplatin and mustine. In addition, we have studied the formation of the different classes of cisplatin–DNA adducts. We have also compared the intracellular accumulation of platinum in the two cell lines following exposure to cisplatin, to find out whether the difference in drug sensitivity is caused by a difference in cellular cisplatin uptake. Finally, the levels of glutathione both in whole cells and in isolated nuclei have been compared.

MATERIALS AND METHODS

Cells

Two cell lines established from human melanoma metastases, RPMI 8322 [26] and A 375 [27] were obtained from Prof. Å. Espmark, National Bacteriological Laboratory, Stockholm, Sweden. Both cell lines were cultured in Eagle's MEM with Earle's salts (Flow), supplemented with 2 mmol/l L-glutamine, 10% fetal calf serum (FCS), 125 IU/ml benzylpenicillin and 125 μg/ml streptomycin. L1210 mouse leukaemia cells were cultured in RPMI1640 medium with HEPES buffer (Flow) supplemented as above.

Drugs and chemicals

Cisplatin was a generous gift from Bristol Laboratories, Syracuse, New York. Immediately before drug incubation 2 mg cisplatin was dissolved in 50 μl dimethylsulphoxide and diluted to the appropriate concentrations in cell culture medium without FCS. Melphalan was obtained as a sterile powder from the Wellcome Foundation, London. Stock solutions were prepared by dissolving 100 mg of melphalan in 1 ml 92% ethanol with 2% HCl and diluting with 9 ml 60% propylene glycol, 1.2% K_2HPO_4 in sterile water. These stock solutions were immediately frozen and stored at −70°C in aliquots of 2 mg melphalan. Stock solutions were renewed every 3 months. Immediately before drug incubation a stock solution was further diluted in cell culture medium without FCS to the desired drug concentrations. Mustine was obtained as a sterile powder from the Boots Company, Nottingham, UK. Immediately before drug incubation 10 mg mustine was dissolved in 10 ml cell culture medium without FCS and further diluted to the appropriate concentrations. [methyl-^{14}C]-thymidine (2.22 GBq/mmol, 1.85 MBq/ml) and [methyl-^{3}H]-thymidine (185 GBq/mmol, 37 MBq/ml) were obtained from the Radiochemical Centre, Amersham, UK.

Drug-induced cytotoxicity

Drug-induced cytotoxicity was measured as the inhibition of colony formation. Appropriate numbers of cells were seeded into 6 cm diameter Petri dishes with 4 ml Eagle's MEM with 10% FCS and 2 mmol/l L-glutamine, and allowed to attach to the bottom overnight. The cells were then exposed to various concentrations of drug for 30 min in medium without FCS. In each experiment triplicate dishes of drug-treated as well as untreated control cells were included. After removal of the drug the cells were grown in fresh medium with 10% FCS and 2 mmol/l L-glutamine for 14 days to produce colonies of appropriate size. The dishes were then rinsed with phosphate-buffered saline (PBS), fixed with formaldehyde and stained with Giemsa. Colonies containing at least 50 cells were counted. The plating efficiency for each dish was calculated as the ratio of the number of colonies over the number of cells plated. For each drug dose the surviving fraction was calculated as the ratio of the mean plating efficiency in dishes containing drug-treated cells over the mean plating efficiency in control dishes with untreated cells.

Drug induced DNA crosslinking

The alkaline elution technique developed by Kohn *et al.* [28] was used with minor modifications as described previously [29, 30]. DNA of RPMI 8322 and A 375 melanoma cells was labeled by growing cells in medium containing ^{14}C-thymidine (37–74 kBq/ml), for 24 h. DNA of L210 cells, to be used as an internal standard in the assays, was similarly labeled by growth in medium containing ^{3}H-thymidine (37 kBq/ml). Following this, the melanoma cells were incubated for 30 min with various concentrations of drug in culture medium without FCS. After removal of the drug the cells were further incubated in drug-free medium with 10% FCS for various lengths of time. The melanoma cells were then irradiated with 6 Gy and the L1210 cells with 3 Gy using a Siemens Stabilopan orthovoltage X-ray machine operating at 140 kV. During and after irradiation the cells were kept on ice in cold PBS with 2% FCS to prevent repair of X-ray-induced DNA strand breaks. Melanoma cells and L1210 cells (approximately 0.2×10^6 of each type) were then collected on polycarbonate filters (pore size 2 μm, diameter 25 mm, Nucleopore, Pleasanton, California), lysed with a 2% sarkosyl-0.02 mmol/l EDTA solution, pH 9.5, and treated with 0.5 mg/ml proteinase K (Merck, Darmstadt, Germany) for 1 h. The DNA was then eluted with a 0.02 mmol/l EDTA solution containing 0.1% sarcosyl, adjusted to pH 12.1 with tetraethylammonium hydroxide as previously described [29, 30]. The elution was performed for 16 h at a rate of 0.035 ml/min, and the eluate collected in fractions. The fraction

of ^{14}C-labeled DNA from melanoma cells that was retained on the filter at the time when 25% of the ^{3}H-labeled DNA from L1210 cells remained on the filter was calculated for each sample. The amount of DNA interstrand crosslinks was then calculated from the following formula [28]:

$$CLF = \sqrt{\frac{1-r_0}{1-r}} - 1$$

where CLF is the crosslinking factor, r is the fraction of ^{14}C-DNA from drug-exposed irradiated cells and r_0 the fraction of ^{14}C-DNA from irradiated control cells retained on the filter. By multiplying CLF with the dose of irradiation (6 Gy), the amount of DNA interstrand crosslinks is expressed as Gy equivalents.

Analysis of cisplatin–DNA adducts with ELISA

RPMI 8322 and A 375 cells (10^6 cells/ml) were treated for 30 min with various doses of cisplatin in Eagle's MEM without FCS. After the drug incubations cells were washed once in PBS, centrifuged, and the cell pellets immediately snap-frozen in liquid nitrogen. After thawing of the samples, DNA was isolated and enzymatically digested to unmodified mononucleotides and platinum-containing (di)nucleotides, followed by chromatographic separation and determination of the latter products with polyclonal antibodies as described elsewhere [31]. Briefly, the DNA digest was chromatographed on the anion-exchange column MonoQ (Pharmacia, Uppsala). The cisplatin adducts were then quantified by assaying the appropriate column fractions in the competitive ELISA.

The chromatograms of the digested DNAs isolated from the A 375 cells indicated that these DNA samples were contaminated with RNA (not shown); approximately 20% of the nucleotides in the digests originated from RNA. Because RNA reacts with cisplatin more readily than DNA, this contaminating RNA will have had a higher relative adduct content than the DNA. According to Pascoe and Roberts [32], inside cells the difference in reactivity results in an adduct level in RNA that is five times that of DNA. The fate of the RNA adducts during nucleolytic digestion and subsequent separation was studied in a separate experiment. Platinated RNA yielded an adduct elution pattern very similar to that of cisplatin-treated DNA, according to analysis by atomic absorption spectroscopy (not shown). The same retention times were obtained for the ribo adducts compared with the deoxy adducts as was proven by cochromatography of digested RNA and DNA samples. The recognition by the antibodies of RNA adducts in the competitive ELISA was comparable to that found for the DNA adducts. In view of these results, proper correction of the DNA adduct data for the RNA contamination could be obtained, by multiplication with a factor of 4/9.

Cellular accumulation of platinum

Melanoma cells were grown overnight in Eagle's MEM with 2 mmol/l L-glutamine, 10% FCS and antibiotics in 50 ml test tubes. Following the removal of 4 ml of each cell suspension for protein analysis according to Lowry *et al.* [33], the cells were exposed to cisplatin in Eagle's MEM without FCS for 30 min. Exposure was terminated by placing the cell suspensions on ice, spinning the cells down, removing the drug-containing culture medium and washing the cells once in drug-free medium. The cells were then spun down and the pellets allowed to dry. Before analysis the cell pellets were resuspended in 200 µl distilled water and 50 µl 10% HCl and sonicated. The cell material as well as samples of the drug-containing culture medium were then analysed for platinum content with flameless atomic absorption spectroscopy using a Pye Unicam atomic absorption spectrophotometer PU 9000 at Analytica AB, Täby, Sweden. The platinum content of each sample was related to the protein content, to correct for differences in cell numbers, as well as differences in cell size between the two cell lines.

Determination of glutathione levels in whole cells and isolated nuclei

Melanoma cells were grown as described above. Nuclei were isolated according to Nüsse and Kramer [34], with minor modifications, as previously described [35]. Briefly, cells were treated for 20 min with a hypotonic solution (585 mg/l NaCl, 1.0 g/l Na citrate, 0.3 ml/l Nonidet P40), after which 1.5 ml 0.25 mol/l sucrose containing 1.5% citric acid was added. After 10–15 min two washes in hypotonic solution without detergent were performed. The content of glutathione in whole cells and isolated nuclei was determined by the method of Tietze [36] as modified by Griffith [37], except that 5% trichloroacetic acid in 12.5 mmol/l EDTA was used for extraction. The glutathione contents were related to the protein contents of the cell samples as determined according to Lowry *et al.* [33].

RESULTS

Drug-induced cytotoxicity

Figure 1 illustrates the survival of the two melanoma cell lines following exposure to the different drugs. A 375 cells were 3-fold as sensitive to cisplatin as RPMI 8322 cells, as determined by comparison of the drug concentrations required to reduce cell survival to 10%. Similarly, A 375 cells were 1.5-fold as sensitive as RPMI 8322 cells to mustine. In contrast, the A 375 cells were 1.5-fold as resistant to melphalan compared to RPMI 8322 cells.

Drug-induced DNA crosslinking

The build up of DNA crosslinks following exposure of cells to cisplatin is a delayed process [6, 17, 21, 22, 25]. DNA interstrand crosslinks were therefore measured 6 h after exposure of RPMI 8322 and A 375 cells to cisplatin (Fig. 2). The cisplatin-sensitive A 375 cells showed levels of DNA crosslinks twice as high as those of RPMI 8322 cells [the ratio (S.D) of slopes of regression lines was 2.0 (0.1)]. In contrast to cisplatin, mustine induces DNA crosslinks rapidly [29]. When DNA interstrand crosslinks were measured immediately following mustine exposure, A 375 cells had higher levels than the more resistant RPMI 8322 cells (Fig. 2). Thus, for both drugs, the more sensitive cell line showed higher levels of DNA interstrand crosslinks.

Analysis of cisplatin–DNA adducts with ELISA

The levels of the cisplatin–DNA adducts in the cells were determinated by immunochemical detection of the specific platinum-containing fragments that resulted after enzymatic digestion of the DNAs isolated from these cells. The results of the assays are given in Table 1; they show an almost linear increase with cisplatin dose. In both cell lines the majority of the adducts are the intrastrand crosslinks. In the procedure applied, these yielded the products Pt-GG (i.e. platinum bound to two neighboring guanines), Pt-AG derived from cisplatin chelated on the bases in the sequence pApG, and G-Pt-G which derived from cisplatin bound to two guanines separated by one or more bases. In addition some G-Pt-G was obtained that was derived from interstrand crosslinks on guanines, a type of adduct amounting

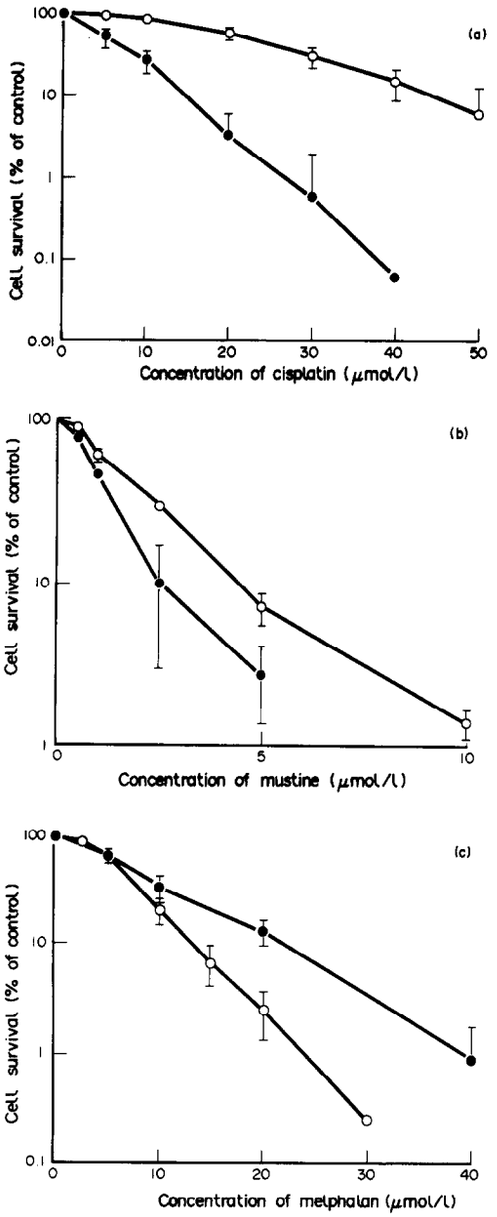

Fig. 1. Cytotoxicity of (a) cisplatin, (b) mustine and (c) melphalan to RPMI 8322 (○) and A 375 cells (●). Cell survival was measured by colony formation. Symbols indicate mean values of 2–8 separate experiments; bars indicate S.E.

to about 1% of the total DNA platination. Finally, Pt-G, the digestion product from cisplatin monofunctionally bound to guanine, formed.

The values for the A 375 cells were obtained with DNA samples contaminated with about 20% RNA, which also reacts with cisplatin. For a comparison of these results with those of the RPMI 8322 cells, a correction for the presence of the cisplatin RNA adducts had to be applied (see Materials and Methods). In Fig. 3 these corrected data for total cisplatin–DNA adducts are shown together with the RPMI 8322 values. The ratio between the slopes of the lines is 2.6, indicating that in the A 375 cells about 2.6 times as many DNA adducts are formed compared with RPMI 8322 cells during incubation with cisplatin. This is in good agreement with the 2-fold higher level of DNA interstrand crosslinks in these cells and the 3-fold difference in sensitivity following cisplatin treatment.

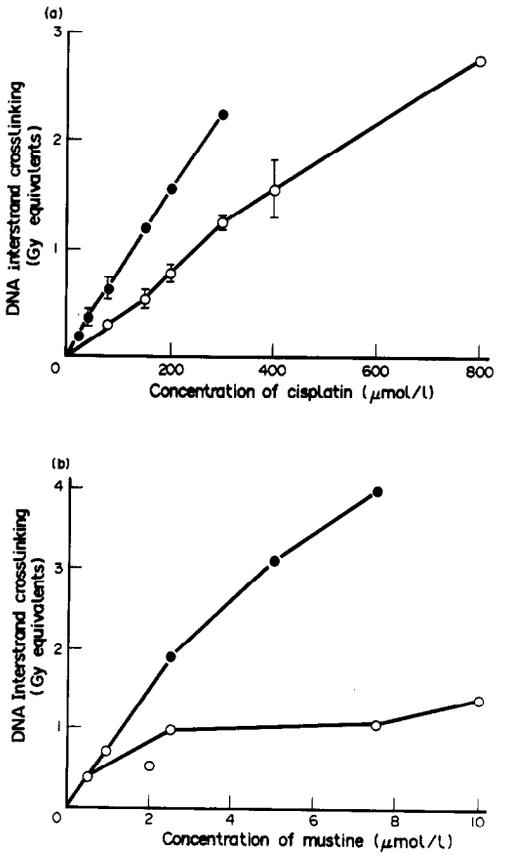

Fig. 2. DNA interstrand crosslinking in RPMI 8322 (○) and A 375 (●) cells measured (a) 6 h following exposure to cisplatin and (b) immediately following exposure to mustine. Symbols indicate mean values of 2–4 separate experiments; bars indicate S.E.

Cellular accumulation of platinum

Measurements of cellular accumulation of platinum following exposure to several different concentrations of cisplatin showed almost linear increases in platinum content following increasing doses of cisplatin (Fig. 4). The measurements gave very similar results for both cell types. Consequently, the higher sensitivity

Table 1. Determination of cisplatin–DNA adducts with ELISA

Cisplatin dose (μmol/l)	DNA adducts (fmol/μg DNA)								
	Pt-G†	%*	Pt-Ag	%*	Pt-GG	%*	G-Pt-G	%*	
RPMI 8322									
10	ND‡	0	0.57 (0.08)	20	1.6 (0.0)	56	0.70	(0.32)	24
20	ND‡	0	1.2 (0.1)	23	2.9 (0.1)	56	1.1	(0.1)	21
40	2.0	11	3.6 (0.3)	20	8.1 (0.0)	46	4.1	(0.0)	23
A 375									
10§	5.4	19	4.5 (0.4)	16	14.1 (1.1)	49	5.0	(0.2)	17
20§	9.0	14	7.5 (1.0)	11	23.3 (0.8)	37	24.3	(3.1)	38
40§	15.6	15	23.6 (4.3)	22	49.1 (3.4)	47	17.0	(0.3)	16

Values are mean (range) values of two independent ELISAs.
* Proportion of each type of DNA adduct as a percentage of the total amount of cisplatin–DNA adducts.
† Determined in a single ELISA assay.
§Sample contaminated with RNA.
ND = Not detectable.

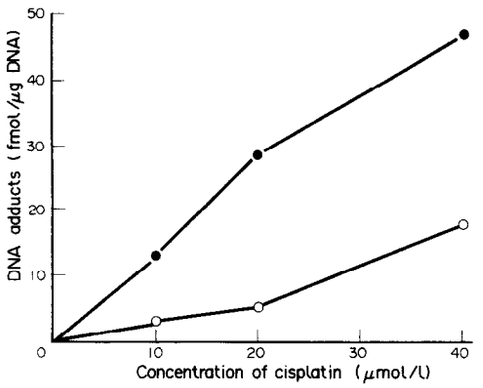

Fig. 3. Total cisplatin–DNA adducts in RPMI 8322 (○) and A 375 cells (●) in relation to drug concentration. The values for A 375 cells have been corrected for the presence of RNA adducts.

and the increased levels of DNA lesions found for A 375 cells was not caused by an increased uptake of cisplatin.

Glutathione levels in whole cells and nuclei

The results of repeated measurements of glutathione levels in whole cells and isolated nuclei are summarised in Table 2. We found that RPMI 8322 cells have an approximately 1.4-fold higher cellular glutathione level. Measurements on isolated nuclei showed a larger difference between the cell lines with a 2.4-fold higher glutathione level in RPMI 8322 nuclei. These measurements were always peformed in parallel with both cell types, and the difference between cell types in nuclear glutathione levels was reproducible. Some loss of glutathione may have taken place during the preparation of cell nuclei, which may have reduced the measured glutathione values. However, any loss of glutathione was likely to occur to a similar extent from the nuclei of both cell types, and the relative proportions of nuclear glutathione should consequently not have been altered. Therefore, the higher glutathione values obtained in the nuclei of RPMI 8322 cells are likely to reflect a real difference in nuclear glutathione concentration rather than experimental artefact.

DISCUSSION

The aim of the present study was to investigate some of the factors responsible for resistance of human tumours to

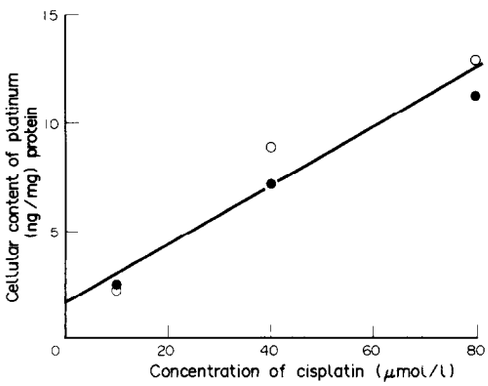

Fig. 4. Cellular accumulation of platinum following exposure of RPMI 8322 (○) and A 375 cells (●) to various concentrations of cisplatin. Platinum content was related to the amount of protein in each sample. Symbols indicate results of a single determination.

Table 2. Glutathione levels in whole cells and isolated nuclei

	Glutathione content (nmol/mg protein)		
	RPMI 8322	A 375	Ratio RPMI 8322: A 375
Whole cells	63.73 (3.64) (n = 9)	46.48 (4.29) (n = 9)	1.37 ($P < 0.01$)
Isolated nuclei	1.02 (0.24) (n = 6)	0.42 (0.09) (n = 6)	2.43 ($P < 0.05$)

Values are given as means (S.D.).
n = number of experiments.
P values were calculated by Student's t test.

chemotherapy with cisplatin. We compared two human melanoma cell lines with differential sensitivity to cisplatin. When measured with a clonogenic assay the cell line A 375 was 3-fold as sensitive to cisplatin as RPMI 8322 (Fig. 1). The A 375 cells were also 1.5-fold as sensitive to mustine, but less sensitive to melphalan, as compared to RPMI 8322 cells. The survival curves of RPMI 8322 cells are in good agreement with our previously reported results with this cell line [29, 30]. The differences between the two cell lines regarding sensitivity to cisplatin and mustine were parallelled by similar differences in induction of DNA interstrand crosslinks (Fig. 2). This correlation between cytotoxicity and induction of DNA crosslinks is in agreement with earlier reports on human cells with different intrinsic sensitivities to cisplatin [19–22, 25]. Analysis of platinum–DNA adducts showed a similar distribution of the different types of DNA adducts in both cell lines, but a higher level of lesions in the sensitive A 375 cells (Table 1, Fig. 3). These results indicate that the variation in sensitivity to cisplatin and mustine can be accounted for by a difference in the induction of DNA adducts by the drugs.

Despite the difference in sensitivity and formation of DNA lesions following treatment with cisplatin, there was a similar cellular accumulation of platinum in the two cell lines (Fig. 4), indicating that the drug uptake was approximately equal. We have previously found a similar accumulation of platinum following cisplatin exposure in RPMI 8322 cells and cisplatin sensitive phytohaemagglutinin-stimulated lymphocytes [25]. An increased drug uptake has been described in two cisplatin sensitive testicular cancer cell lines, compared to a more resistant bladder cancer cell line [23]. The relevance of this finding for the difference in drug sensitivity is questionable, however, since the higher cisplatin uptake was not correlated to an increase in DNA adduct formation. Thus, despite the frequent reports of decreased uptake of drug cells with *in vitro* induced resistance to cisplatin [6–12], alterations in drug uptake have so far not been established as an important factor determining the inherent sensitivity of human cells to cisplatin.

Increased levels of glutathione have frequently been observed in cisplatin resistant cells [7, 13–16, 22]. The higher content of glutathione in RPMI 8322 cells (Table 2) could therefore be of importance for the decreased toxic effect of cisplatin on these cells. An increase in cellular glutathione might reduce the cytotoxicity of cisplatin by several possible mechanisms.

First, glutathione could react with and inactivate cisplatin before it reaches the DNA target. Such reactions could possibly be catalysed by GST, in analogy to the demonstration that the bifunctional alkylating agent melphalan is conjugated to

glutathione by a GST mediated reaction [38]. An increased level of GST has been described in a cisplatin-resistant bladder carcinoma cell line [22]. We have previously found that both RPMI 8322 and A 375 cells have high levels of class Pi GST, but no major differences between the cell types in the activities of the GST isoenzymes were seen [39].

Second, the high level of intranuclear glutathione in RPMI 8322 cells (Table 2) may contribute to the inactivation of cisplatin–DNA monoadducts and thus prevent the delayed conversion of monofunctional lesions to DNA crosslinks. It has been shown that glutathione can bind to platinum–DNA monoadducts *in vitro* [40]. Such quenching of monoadducts has been suggested as a mechanism of resistance to cisplatin [17]. However, since the present analyses showed lower levels of all types of platinum–DNA adducts (including Pt-G monoadducts) in RPMI 8322 cells, it is unlikely that the reduced level of DNA interstrand crosslinks is caused by increased quenching of platinum–DNA monoadducts.

Finally, since there is an indication that glutathione may be required for the repair of X-ray induced DNA strand breaks [41], it is conceivable that the increased level of glutathione in the nuclei of RPMI 8322 cells might lead to enhanced repair of cisplatin–DNA adducts. This possibility is supported by the finding that depletion of cellular glutathione by buthionione sulphoximine (BSO) reduces cisplatin-induced DNA repair synthesis in a resistant ovarian carcinoma cell line and sensitises the cells to cisplatin [42]. This is in apparent contradiction to our finding that BSO does not affect the rate of removal of mustine-induced DNA interstrand crosslinks in RPMI 8322 cells [30]. However, an effect of lowered glutathione levels on DNA polymerase activities, either by a direct inhibition of the enzymes or by a decrease in available deoxyribonucleotide triphosphates [42], may not have been detected in our analysis of the rate of removal of DNA crosslinks, which measures an earlier step in excision repair. Also, the lack of effect of BSO on removal of mustine-induced DNA crosslinks could be due to inefficient depletion of nuclear glutathione [35].

The importance of cellular glutathione levels for resistance to cisplatin can be challenged by the fact that depletion of glutathione by BSO typically gives only a low degree of sensitisation to this drug [7, 30]. It is, however, possible that the nuclear glutathione concentration may be more important than the total cellular level of glutathione for resistance to cisplatin. BSO is inefficient in depleting the nuclear levels of glutathione [35]. Prolonged treatment with BSO, which may lead to a more efficient depletion of glutathione from nuclei, can sensitise cells significantly to cisplatin [13, 43]. We therefore compared the glutathione levels in isolated nuclei from the two cell types (Table 2). The lower nuclear glutathione content in A 375 cells may possibly explain the increased sensitivity to cisplatin. The possibility that nuclear glutathione levels, rather than the total cellular levels, are of importance for resistance to cisplatin is a novel hypothesis that should be further tested.

In contrast to the small effect of BSO on cisplatin sensitivity, RPMI 8322 cells are significantly sensitised to mustine by treatment with BSO [30]. The higher cellular GSH levels of RPMI 8322 cells may therefore explain why they are more resistant than A 375 cells to this drug.

The rate of repair of DNA adducts has been shown to be of importance for cellular sensitivity to cisplatin [8, 23]. Xeroderma pigmentosum cells, which are deficient in the repair of cisplatin–DNA adducts, are hypersensitive to this drug [44]. Increased removal of DNA intrastrand crosslinks has been described in cisplatin resistant cells [8], and a decreased rate of removal of such lesions was found in a cisplatin sensitive testicular tumour cell line [23]. The differential sensitivity of our two cell lines to cisplatin can be attributed to different levels of DNA adducts. It is possible that a higher rate of repair of cisplatin–DNA adducts may contribute to the lower levels of DNA lesions in the resistant RPMI 8322 cells. However, a 2.6-fold difference was seen when the number of cisplatin–DNA adducts was compared in the two cell types immediately after a 30 min drug incubation, which would allow little time for DNA repair to modify the outcome in DNA lesions (Table 1, Fig. 3). Moreover, the lower sensitivity of A 375 cells to melphalan shows that these cells are not generally more sensitive to DNA crosslinking agents than RPMI 8322 cells, which would be expected if they were less capable of repairing DNA crosslinks. It is therefore questionable whether a difference in the DNA repair capacity of the cell lines contributes to the difference in cisplatin sensitivity.

Despite the higher sensitivity of A 375 cells to cisplatin and mustine, these cells were more resistant than RPMI 8322 cells to melphalan (Fig. 1c). This confirms that resistance to some bifunctional DNA crosslinking drugs does not inevitably result in crossresistance to all other agents of this class. Rather, separate mechanisms may cause resistance to different bifunctional DNA crosslinking drugs. The cause of the difference in melphalan sensitivity of the two cell lines has not been explored further, but could conceivably be related to drug uptake. Melphalan enters cells by an active process, mediated by leucine carriers, and decreased uptake of melphalan has been described in resistant cells [45, 46].

In summary, we have found that A 375 cells are more sensitive to cisplatin and mustine than RPMI 8322 cells. The differences in sensitivity can be explained by similar differences in drug induced DNA adducts. The differential sensitivity to cisplatin is not due to a difference in drug accumulation. Increased overall cellular levels of glutathione are likely to be of importance for the resistance of RPMI 8322 cells to mustine. The higher concentration of glutathione in the nuclei of RPMI 8322 cells may be important for the relative resistance of these cells to cisplatin.

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Acknowledgements—We thank Eva Grafström, Doris Kröckel, Suzanne Egyhazi, Helma van Dijk-Knijnenburg and Saskia van der Velde-Visser for skilful technical assistance.

This investigation was supported by the King Gustaf V Jubilee Fund, the Swedish Cancer Society, Stockholm, Sweden and the Dutch Cancer Society (grant KWF-MBL 87-1), The Netherlands.