Eur J Cancer, Vol. 29A, No. 4, pp. 549–554, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Effect of Whole Body Hyperthermia on *Cis*-Diamminechloroplatinum (II)-induced Antitumour Activity and Tissue Pt-distribution: Do Anaesthetics Influence the Therapeutic Ratio?

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Thermal enhancement of *cis*-diamminedichloroplatinum (II) (DDP)-mediated antitumour activity and normal tissue toxicities by whole body hyperthermia were compared in a F344 rat model under different anaesthetic conditions. Whole body hyperthermia (WBH: 120 min at 41.5°C) enhanced both DDP-mediated antitumour activity and toxic side-effects. Our present study shows that anaesthetics might influence the thermal enhancement ratios (TER) calculated for DDP-mediated normal tissue toxicity but did not influence the TER calculated for antitumour activity. The TER calculated for DDP-mediated antitumour activity was 2.9. As a result of the anaesthetics used, the TER calculated for kidney and gastrointestinal toxicity ranged from 1.8 to 4.5 and from 1.2 to 2.3, respectively. The TER estimated for DDP-mediated general toxicities varied between 2.9 and 4.0 for weight loss, and from 2.0 to 2.3 based on the LD_{50}. The differential effect of anaesthetics on TER calculated for antitumour activity and normal tissue toxicity led to different therapeutic ratios. For example the therapeutic ratio for combined WBH and DDP, using kidney damage as an end-point for normal tissue damage, ranged from 0.6 (without anaesthesia) to 1.6 (using nembutal as anaesthetic). The significantly elevated platinum levels in serum, kidney, jejunum and tumour tissue after WBH treatment may explain the thermal enhancement of DDP-mediated antitumour activity and side-effects but no correlation could be found for the differences in DDP-mediated normal tissue toxicities induced by the anaesthetics.
Eur J Cancer, Vol. 29A, No. 4, pp. 549–554, 1993.

INTRODUCTION

Cis-DIAMMINEDICHLOROPLATINUM (II) (DDP) has been shown to display a potent antitumour activity [1] that is increased when it is combined with hyperthermia [2–6]. Discrepancies are reported for the sensitivity of tissues and experimental tumours treated with DDP used in combination with hyperthermia [4, 6–10]. The agents used to keep the animals anaesthetised during the treatment may play a significant role in causing these differences.

In experimental oncology, anaesthesia is often required to immobilise the animals for different procedures, such as drug administration, pharmacokinetic studies, hyperthermia, radiotherapy and surgery. This is necessary, for instance, to minimise stress and discomfort as a result of the treatment, as is the case with injections, surgery, whole body hyperthermia, kidney function studies (glomerular filtration rate), or for exact positioning of the animal in order to apply local hyperthermia or irradiate parts of the body.

It is known that anaesthetics can have cardiovascular effects [11–13]. For instance, nembutal induces changes in the tumour blood flow and hence may influence the uniformity of tumour heating during hyperthermia [14]. It is also reported that anaesthetics such as ketamine may alter the heating up time in Sprague–Dawley rats during exposure to 2.8-GHz radiofrequency radiation [15]. These physiological changes induced by anaesthetics may lead to changes in pharmacokinetics of anticancer drugs and thereby affect antitumour efficacy [16, 17] or severity of the toxicities [10]. Similarly, nembutal can also alter the radiosensitivity of tumours [18–20] or normal tissues [21, 22].

We recently reported that in rats, anaesthetics strongly influenced the severity of DDP-mediated side-effects both at 37°C and during whole body hyperthermia (WBH) [10]. The present study was undertaken, to examine possible effects of anaesthetics on DDP-mediated antitumour activity both at normal (37°C) and elevated (41.5°C) temperatures, and to compare the antitumour data with the toxic effects after the different treatments. To better understand the interactions of heat, DDP and anaesthetics on tumour as well as normal tissues we also determined the platinum tissue distribution and plasma concentration immediately after treatment.

MATERIALS AND METHODS

Animals

Female Fisher 344 rats (140–170 g) were obtained from Harlan, Sprague–Dawley Inc. (Houston, TX). Animals were fed a diet of standard laboratory chow and allowed water *ad libitum*. They were housed five to a cage and maintained in a controlled environment with a 12-h light/dark cycle. The rats were anaesthetised about 60 min prior to DDP administration. The dosage of the anaesthetic agents was sufficient to maintain

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Revised 11 Sep. 1992; accepted 30 Sep. 1992.

the animals under deep anaesthesia for at least 3 h without causing lethality (for more details see Wondergem *et al.* [10]).

Anaesthetic combination. The rats were anaesthetised using a combination of 3 to 3 to 1 parts (v/v/v) ketamine, xylazine and acepromazine delivered intramuscularly (i.m.). The final concentrations injected were 38 mg/kg ketamine, 8 mg/kg xylazine and 1 mg/kg acepromazine.

Nembutal. Pentobarbital sodium was administered intraperitoneally at a dose of 30 mg/kg.

Halothane. Inhalation anaesthesia was induced by a 3–4% mixture of halothane in oxygen. For maintenance of anaesthesia during the experiment, the halothane concentration was reduced to about 1%.

DDP

DDP was obtained from Bristol Myers (Syracuse, NY). DDP was prepared for administration by reconstitution in sterile water resulting in a final concentration of 1 mg/ml DDP in 0.9% NaCl. DDP was administered intravenously (i.v.) by bolus injection via the lateral tail vein (between 14.00 and 16.00 h).

WBH

WBH was induced by immersing the animal into a thermostatically controlled water bath maintained at a temperature of 41.5°C [6]. An average of 30 min was required for the core temperature of the rats to reach 41.5°C, which was then maintained for 2 h. In rats treated with combined WBH + DDP, DDP was given simultaneously with heat when rectal temperature reached 41.4°C.

Antitumour studies

A transplantable fibrosarcoma (RFSa), originally induced by methylcholanthrene, was used [23]. The tumour is slightly antigenic, moderately well differentiated and has a volume doubling time of ~2 days. The macroscopic growth was logarithmic up to a volume of 5 cm^3 [24]. To obtain tumours, 10^6 viable RFSa cells were injected subcutaneously into the left flank of each rat as described previously [6]. When tumours reached the desired volume (0.3–0.4 cm^3), they were treated with DDP alone, WBH alone or DDP combined with WBH using different anaesthetic regimens. The response of the tumour to the various treatments was studied by determining the parameter of tumour growth delay (TGD). The TGD was calculated at 10 × treatment volume [6, 24].

Platinum determination

Plasma and tissues for platinum determination were obtained at 2 h after DDP administration (= immediately after termination of WBH). Part of the plasma was treated with trichloracetic acid (TCA) to determine the free-platinum fraction in the supernatant as described by Siddik *et al.* [25]. Plasma and the TCA-soluble fraction were analysed directly for platinum content. Portions (about 200 mg) of the right and left kidneys, jejunum and tumour, however, required solubilisation in hyamine hydroxide as described by Siddik *et al.* [26] prior to analysis by flameless atomic absorption spectrophotometry using a Varian Model 1475 AA equipped with an autosampler and a Varian GTA-95 carbon rod furnace (Varian Associates, Sunnyvale, California).

Statistics

ED_{50}* and LD_{50} values (Table 1) leading to a specified level of toxicity at day 5 post-treatment, or to death of 50% of the treated animals within 30 days post-treatment, were calculated from incidence curves using logistic regression analysis (BMDP Statistical Software Inc.).

Statistical differences in TGD values (Fig. 1), and tissue platinum levels (Table 2) between groups (at 37, at 41.5 and 37°C vs. 41.5°C) were calculated using the two-tailed Student's

*Table 1. ED_{50} values (dose of DDP mg/kg) calculated for DDP-mediated acute toxicities (at day 5) for rats treated with DDP alone or combined with whole body hyperthermia (WBH: 120 min at 41°C) using different anaesthetics**

Treatment	Anaesthetic	Kidney BUN	Diarrhoea	General toxicities: Weight loss	General toxicities: LD_{50}
DDP alone	None	6.7 ± 0.7†^{c,d}	7.4 ± 0.4^{d}	6.0 ± 0.1	7.4 ± 0.5^{c,d}
	Halothane	6.1 ± 0.3^{d}	7.5 ± 0.4^{d}	5.9 ± 0.5	7.2 ± 0.6^{c,d}
	Nembutal	4.6 ± 0.4^{a,b,d}	6.2 ± 0.8^{a,b,d}	4.6 ± 1.0^{a}	6.1 ± 0.4^{a,b,d}
	Combination	8.9 ± 0.3^{a,b,c}	9.2 ± 0.5^{a,b,c}	5.7 ± 1.2	9.2 ± 0.5^{a,b,c}
DDP+WBH‡	None	1.5 ± 0.3^{b,c,d}	3.2 ± 0.8^{d}	1.5 ± 1.0	3.6 ± 1.0
	Halothane	2.0 ± 0.2^{a,d}	3.9 ± 0.2^{d}	2.0 ± 0.2	3.2 ± 0.5^{d}
	Nembutal	2.5 ± 0.7^{a}	5.2 ± 1.6^{a}	1.3 ± 0.9	2.8 ± 0.5^{d}
	Combination	2.9 ± 0.2^{a,b}	5.2 ± 0.5^{a,b}	2.0 ± 0.2	4.7 ± 0.7^{b,c}

*The data points used to calculate the ED_{50} values are derived from incidence curves recalculated from dose–response curves such as presented in Wondergem *et al.* [10]. At each incidence curve, 25–35 rats were used for each experimental condition.

†The 95% confidence limits were determined using BMDP (Statistical Software Inc.).

‡ED_{50} values of all DDP + WBH groups are significantly different from DDP alone, when tested for each experimental condition.

[a]Significantly different as compared with the non-anaesthesia group.

[b]The halothane group.

[c]The nembutal group.

[d]The combination group.

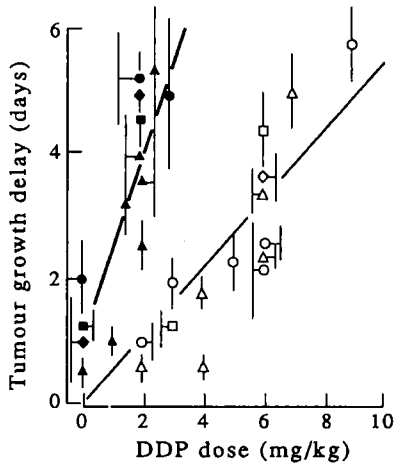

Fig. 1. Tumour growth delay (days) as a function of cisplatin (DDP) dose (mg/kg body weight). Animals received DDP at 37°C (open symbols), or DDP combined with whole body hyperthermia (WBH: 120 min at 41.5°C) (closed symbols). Animals were anaesthetised with either a combination of ketamine (38 mg/kg), xylazine (8 mg/kg) and acepromazine (1 mg/kg) (○, ●), halothane (1%) (△, ▲), or nembutal (30 mg/kg) (□, ■), or without anaesthesia (◇, ◆). Each point represents the mean ± S.E.M., 5–15 animals per data point (data from several experiments).

Table 2. Summary of thermal enhancement ratios (TER) calculated for DDP-mediated acute toxicities under different anaesthetic conditions*

			General toxicities	
Anaesthetic	Kidney BUN	Diarrhoea	Weight loss	LD_{50}
None	4.5 ± 0.9[b,c,d]	2.3 ± 0.6[c]	4.0 ± 2.7	2.1 ± 0.6
Halothane	3.1 ± 0.4[a,c]	1.9 ± 0.3[c]	3.0 ± 0.2	2.3 ± 0.4
Nembutal	1.8 ± 0.5[a,b,d]	1.2 ± 0.4[a,b,d]	3.5 ± 2.5	2.2 ± 0.4
Combination	3.2 ± 0.2[a,c]	1.8 ± 0.2[c]	2.9 ± 0.4	2.0 ± 0.3

*The TER values presented in this table are derived from data points presented in Table 1.
[a]Significantly different as compared with the non-anaesthesia group.
[b]The halothane group.
[c]The nembutal group.
[d]The combination group.

t-test. A *P*-value < 0.05 was considered to be statistically significant.

The thermal enhancement ratio (TER) was calculated as follows:

$$\text{TER} = \frac{\text{DDP dose without WBH that caused a specified effect}}{\text{DDP dose with WBH causing the same specified effect}}.$$

The estimate of TER for DDP-mediated antitumour activity was expressed as the ratio of slopes of the dose–response curves. The curves (Fig. 1) were plotted by means of linear regression (Graphpad software, ITI Press, Philadelphia, PA).

The therapeutic ratio was calculated as follows:

$$\text{Therapeutic ratio} = (\text{TER}_{\text{antitumour activity}}/\text{TER}_{\text{toxicity}}).$$

Therapeutic gain is concluded when the therapeutic ratio > 1.0. Conversely, therapeutic loss occurs at a ratio < 1.0.

RESULTS

Tumour studies

Figure 1 shows a dose-dependent increase of the tumour response after DDP administration. At 37°C no significant effect of the anaesthetics on the DDP-mediated tumour growth delay could be observed when tested at a DDP dose of 6 mg/kg. WBH treatment alone (120 min at 41.5°C) caused a minor tumour response (TGD of 0.5–2.0 days). However, when WBH was combined with DDP a sharp increase in the tumour response was observed. Again no influence of the anaesthetics on the dose–response relation could be observed. Regression analysis of the "pooled" data presented in the dose–response curves suggested a linear fit (the correlation coefficient calculated for the curves was > 0.90). The slopes (±S.E.M.) for the dose–response curves of DDP with and without WBH were 1.53 (±0.29) and 0.53 (±0.09), respectively. This difference between the slopes was highly significant. For this experimental tumour system a TER of 2.9 (±0.7), based on the ratio of the slopes, was calculated.

Toxicities

In contrast to the tumour studies, the use of anaesthetics led to differences in DDP-mediated toxicities both at 37°C and during WBH (Wondergem *et al.* [10]). Table 1 shows ED_{50} and LD_{50} values calculated for different DDP-mediated toxicities for rats treated with DDP alone or combined with WBH. The ED_{50} values for renal, intestinal injury and lethality at 37°C, using nembutal as anaesthetic, proved to be significantly lower as compared to all other experimental groups. This indicated that the use of nembutal during DDP administration led to an increase in DDP-mediated toxicities. DDP-mediated toxicity using halothane was similar to the toxicities observed in the non-anaesthetised group. The use of the combination anaesthetic clearly reduced the DDP-mediated side-effects as compared to all other experimental conditions.

When WBH was combined with DDP both ED_{50} and LD_{50} values decreased indicating thermal enhancement of DDP-mediated toxicities. Again, anaesthetics influenced the data; however, the number of groups which differed significantly was decreased. Group comparison showed that the combination anaesthetic led to significant lower kidney and gastrointestinal toxicities (vs. the non-anaesthesia group and the halothane group; see Table 1).

The TER calculated for DDP-mediated kidney damage [elevation of blood urea nitrogen (BUN) concentration] under the different anaesthetic conditions varied from 1.8 for nembutal, and from 3.1 to 4.5 for the other experimental conditions (Table 2). The TER calculated for gastrointestinal damage were also influenced by the different anaesthetics (the calculated TER ranged from 1.2 to 2.3). The TER values calculated for renal and intestinal injury using nembutal anaesthesia were significantly lower as compared to all other experimental groups. This table further shows that treating the animals with WBH+DDP without anaesthesia led to a significantly increased TER value

*The ED_{50} value for functional kidney damage was calculated at a blood urea nitrogen (BUN) level of 150 mg/dl (about 10× normal value), whereas the ED_{50} for gastrointestinal damage was calculated at a score of 3.0 (60% of the ventral area of the animal covered with discharge). The ED_{50} for general toxicities was calculated at a weight loss of 10%. Body weight and BUN determinations were performed at day 5 (the time of maximally observed acute toxicity [6, 10]) after treatment. Maximum diarrhoea after administration of DDP alone or combined with WBH was observed 3–6 days after treatment.

Table 3. Effects of anaesthetics on tissue platinum levels 2 h after administration of cisplatin (6 mg/kg DDP, i.v.) at normal temperatures and during whole body hyperthermia (WBH: 120 min at 41.5°C)

		Platinum concentrations (μg/g or μg/ml)					
		Plasma		Kidney			
Treatment	Anaesthetic	Total	% bound	Right	Left	Jejunum	Tumour
DDP alone	None	1.3 ± 0.1*	94.4 ± 2.7	10.9 ± 1.8	11.2 ± 1.8	5.6 ± 0.4	1.8 ± 0.2
	Halothane	1.5 ± 0.1	96.1 ± 1.0	11.7 ± 0.7	12.4 ± 1.8	5.6 ± 0.5	1.6 ± 0.3
	Nembutal	1.5 ± 0.2	94.0 ± 4.7	13.3 ± 2.6	14.1 ± 1.4	5.6 ± 0.4	1.9 ± 0.1
	Combination	1.4 ± 0.2	96.4 ± 1.1	10.6 ± 1.8	11.4 ± 0.6	6.2 ± 0.6	1.9 ± 0.2
DDP+WBH†	None	2.1 ± 0.3	91.5 ± 3.0‡	19.1 ± 3.7	22.8 ± 2.7	7.3 ± 0.7	3.7 ± 0.9
	Halothane	2.2 ± 0.3	94.3 ± 3.0‡	19.7 ± 1.7	24.3 ± 4.4	7.5 ± 0.9	2.8 ± 0.2
	Nembutal	2.2 ± 0.3	90.1 ± 4.6‡	21.0 ± 2.2	21.1 ± 4.3	6.7 ± 0.6	2.8 ± 0.3
	Combination	2.1 ± 0.1	92.6 ± 3.6‡	16.6 ± 2.9	18.8 ± 0.5	6.8 ± 0.9‡	2.6 ± 0.3

*Values are presented as the mean ± S.D. of 4 animals.
†Cisplatin levels of DDP+WBH groups (pooled data from all experimental conditions) are significantly different from the DDP alone groups.
‡Not significantly different from DDP alone when tested for each experimental condition; platinum levels of all other WBH+DDP groups are significant from DDP alone.

for kidney damage. The TER calculated for weight loss and lethality were hardly influenced by the anaesthetics, and no significant differences between the calculated TER could be observed. The TER calculated for LD_{50} values only varied a little (2.0–2.3).

Platinum determination

At 37°C, the amount of platinum present in tumours with a volume of 0.3–0.4 cm^3, kidneys, jejunum and plasma, 120 min after i.v. injection of 6 mg/kg DDP, was not influenced by the use of different anaesthetics (Table 3). For all treatment groups, platinum content in the kidneys was highest, whereas the platinum content of plasma and tumour were lowest. Platinum levels in the left kidney were always slightly higher than in the right kidney. However, this difference was not significant. Moreover, when tested for each tissue no significant effect of the different anaesthetics on the platinum levels could be observed.

When DDP (6 mg/kg) was administered simultaneously with WBH, platinum levels in all tissues studied were significantly elevated compared to the 37°C group. The average increase of platinum levels in the tumour was 65%, and in the kidney this difference was almost 71%. Platinum levels in plasma and jejunum were increased by only 48 and 24%, respectively. The percentage of bound platinum in plasma was slightly but significantly lower as compared to the 37°C group. Also at elevated temperatures, statistical testing did not reveal any effect of the anaesthetics on the platinum levels.

DISCUSSION

Our data demonstrate that WBH alters DDP-mediated antitumour activity, side-effects, and platinum distribution in different tissues. Independent of the anaesthetic status of the rat, the large increase of DDP-mediated antitumour activity and normal tissue effects after combined WBH+DDP seemed to match with the significantly elevated platinum levels in tumour, serum, kidneys and jejunum, when compared with normothermic animals.

No clear relation between anaesthetic-induced changes in tissue platinum levels and alterations in the severity of side-effects could be found in this rat model, in spite of the fact that anaesthetics clearly influenced DDP-mediated toxicities both at normal and elevated temperatures. Examination of tissue platinum levels 2 h after DDP administration revealed no significant differences in tissue drug content induced by the use of the anaesthetics either in the normothermic groups or in the hyperthermic groups. This observation suggests that the effects of anaesthetics on DDP-mediated side-effects could not be explained by differences in platinum distribution during the treatment. Possibly physiological changes after completion of the treatment were responsible for the observed differences in toxicity.

The increased platinum levels, measured immediately after hyperthermia, were likely the result of diminished platinum excretion due to impairment of the renal blood flow during WBH. A shift in total blood flow from the body core, especially the kidneys and intestine, to the periphery has been reported in WBH-treated animals (see review by Buhring and Eggana [27]). Moreover, other experiments from our laboratory showed a reduced urine production or even total anuria in rats after 120 min at 41.4°C WBH treatment [28]. The elevated platinum levels in serum will lead to an increase of platinum in various tissues. However, the degree of elevation of platinum levels differed from tissue to tissue. The average increase of platinum in the tumour and kidney was 65–71%, whereas the average increase of platinum levels in serum and jejunum was only 48 and 24%, respectively. These data may explain the differences in the thermal enhancement ratio (TER) calculated for kidney [average TER = 3.2 (1.8–4.5)] and intestinal damage [average TER = 1.8 (1.2–2.3)] after combined WBH+DDP. In addition to modulation of blood flow to the different tissues at risk during WBH, differences in DDP uptake for the individual tissues may also contribute to these alterations.

Besides the elevated platinum levels in serum and several tissues, we also observed a small but significant decrease of bound platinum in serum after WBH, indicating an increase of free platinum. The average increase, measured for all anaesthetic conditions was about 65%. Our observations were in agreement with those of Riviere *et al.* [29], who studied the effect of heat on DDP pharmacokinetics in normal dogs. They showed *in vivo* alterations of different pharmacokinetic parameters, such as a significant increase in free platinum at 42°C and an increased

cisplatin half-life. Based on their *in vivo* and *in vitro* results they postulated that WBH enhances the tissue binding of free platinum which may lead to enhanced toxicity. This appears to have been confirmed in the present study. Our pharmacokinetic data and those of Riviere *et al.* [29] were different from those reported by Gerard *et al.* [8] in humans. The latter investigator did not observe any alteration in plasma or urinary pharmacokinetics of total and ultrafiltrable platinum as a result of heat in 3 patients treated with combined WBH+DDP. It might be possible that the vigorous hydration (800 ml/h) during heating to maintain blood pressure and urine output at an acceptable level was responsible for this effect. Nevertheless, they observed unacceptable nephrotoxicity as a result of this treatment in all patients.

Thermal enhancement ratios calculated for DDP-mediated kidney and gastrointestinal toxicity varied depending on the anaesthetic used. Differences in TER calculated for weight loss were not significant. The TER calculated for DDP-mediated kidney toxicity from the nembutal group tended to be lower than those for the other anaesthetics. This may be explained by the fact that renal function during nembutal-induced anaesthesia at normal temperature was probably already compromised, and that some physiological mechanisms to maintain minimum renal function prevented an additive effect in renal toxicity by WBH from being realised. Thus, the apparent renal function decrement by WBH in conjunction with nembutal was lower than with halothane or the combination anaesthetic.

The results of this study further indicate that the therapeutic indices ($TER_{antitumour\ act.}/TER_{toxicity}$) calculated for DDP in combination with WBH were influenced by the experimental conditions under which they are measured. For instance, the therapeutic ratio for this combination using nembutal as anaesthetic was 1.6, 2.4, 0.8 and 1.3, when using kidney damage, gastrointestinal damage, weight loss and lethality, respectively, as an end-point for normal tissue toxicity. Under other anaesthetic conditions and without anaesthesia, the therapeutic ratio for DDP+WBH, based on DDP-mediated kidney toxicity was less than unity; 0.6 (no anaesthesia) to 0.9 (either with halothane or the combination anaesthetic). Under the latter experimental conditions one has to conclude that combining this drug with WBH leads to a therapeutic loss. However, one has to keep in mind that this functional end-point for renal damage, a BUN level of 150 mg/dl (about 10× normal value), used to study to DDP-mediated normal tissue toxicity, was determined at day 5 after treatment. The increase of BUN levels (a maximum elevation was observed at 5 days post treatment) in plasma of animals surviving the treatment for a longer period (>3 months) was only transient and returned to near normal levels after a few weeks. When considering lethality (LD_{50}) as the most important end-point, the therapeutic ratio combining DDP with WBH, for all experimental conditions, was between 1.3 and 1.4. Presumably combining DDP with WBH led to a therapeutic gain. Moreover, the small range in therapeutic indices suggests that anaesthetics did not influence the therapeutic ratio using lethality as an end-point. This latter conclusion seems to be in contrast with the conclusion which could be drawn from the therapeutic index using kidney function as an end-point for toxicity. Depending on the anaesthetics used, the therapeutic ratios ranged from 0.6 (no anaesthesia) to 1.6 (nembutal). One has to be very cautious, therefore, to extrapolate such data directly to the clinical situation. Moreover, no information is yet available on the late normal tissue response to the combination of WBH+DDP. In particular, information on chronic renal damage is very important for the design of new clinical trials including combined WBH and DDP treatment. Nevertheless, these studies provide the hyperthermist with important information about the degree of thermal enhancement that can be achieved with combined DDP+WBH.

Further studies which explore the alterations in DDP pharmacokinetics, tissue distribution and excretion during WBH have to be conducted to provide further information in order to improve the therapeutic index of combined DDP+WBH treatment.

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Acknowledgement—This work was supported by N.C.I. Grant: Rol Ca43090.

Eur J Cancer, Vol. 29A, No. 4, pp. 554–558, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

High Rate of Expression of Multidrug Resistance-associated P-Glycoprotein in Human Endometrial Carcinoma and Normal Endometrial Tissue

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The overexpression of P-glycoprotein was studied in 10 normal endometrial controls (five from the proliferative and five from the secretory phase of the menstrual cycle) and in 23 endometrial carcinomas of different histological varieties, using the C219 and JSB-1 monoclonal antibodies. Three of the tumours had been previously treated with combination chemotherapy containing doxorubicin. All endometrial carcinomas, whether treated or untreated, as well as the normal endometrial controls from both the proliferative and the secretory phase of the menstrual cycle, overexpressed P-glycoprotein. This puts endometrial carcinoma into the same category as other tumours arising in organs which normally overexpress P-glycoprotein, all of which tend to be intrinsically resistant to chemotherapy.
Eur J Cancer, Vol. 29A, No. 4, pp. 554–558, 1993.

INTRODUCTION

P-GLYCOPROTEIN, a membrane-bound extrusion pump, is a mediator of multidrug resistance in experimental tumour cells, and seems also to play a role in the resistance to chemotherapy of human tumours [1–3]. Recently, the presence of P-glycoprotein in clinical tumour specimens has been correlated with their relapse rate after chemotherapeutic treatment [4]. More remarkably, the presence of P-glycoprotein has been inversely correlated with the survival of patients independently of the kind of treatment received by them [5]. It thus seems that P-glycoprotein may be of use for clinicians not only for discriminating which tumours will eventually respond or not to a certain kind of chemotherapy, but also as a powerful general prognostic factor. To define these applications of P-glycoprotein measurements in the future, however, detection and evaluation methods must still be optimised, to allow for the comparison of results between different investigators. One of the most widely employed assays for the detection of this and many other tumour markers in the clinical setting is immunohistochemistry, which is gaining acceptance as both a practical and reliable diagnostic method. One of its main advantages, for clinical purposes, over biochemical methods is the preservation of tissue architecture. This allows detection of P-glycoprotein in small cell subsets down to the single cell level, to determine its distribution in both tumoral and normal tissue and to account for the cellular heterogeneity present in every tumour. On the other hand, it is less sensitive than biochemical methods such as RNA and DNA measurements by means of cDNA probes, widely employed by other groups, which, however, are carried out on bulk tissue

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Revised 18 Sep. 1992; accepted 1 Oct. 1992.