grade III or IV tumours [13]. It may be suggestive from this finding that patients classified as stage A or stage B may also benefit from the adjuvant setting with levamisole and 5-FU.

Another intriguing observation is the activity of levamisole/5-FU combination on rectal and sigmoidal adenocarcinoma grafts. Although these tumours have a different behaviour compared with colon carcinoma [14], regression of implants was also demonstrated. Therefore further preclinical and/or clinical evaluation of levamisole in other oncologic settings is warranted to elucidate fully its potential use for the treatment of neoplasms.

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The Influence of Hyperthermia on the Uptake of Cisplatin in the Rat Cervical Spinal Cord

Peter Sminia, Gerrit Los, Jos J.G.W. Hendriks, Marianne J.H. van Vugt, Jaap Haveman and Dionisio González González

The influence of local hyperthermia on the uptake of cisplatin in the rat cervical spinal cord was investigated. After single intraperitoneal or intravenous injection of cisplatin (5 mg/kg body weight), the spinal cord region cervical 5-thoracic 2 was heated for 60 min at mean (S.D.) 41.2 (0.4) °C or 40 min 42.4 (0.3) °C using a 434 MHz microwave heating device. One day after treatment with either hyperthermia alone, cisplatin alone or the combination, none of the animals expressed neurological symptoms. The spinal cord was dissected and platinum levels were measured by flameless atomic absorption spectroscopy. No difference was found in uptake of platinum in the spinal cord between control- and heat treated animals. In a second series of experiments, the spinal cord was heated for 30–60 min. during a 2 h infusion of cisplatin. One day after treatment at 42.3°C for 60 min, neither motor nor sensory functions were affected and platinum levels did not differ significantly between control and treated animals. Also, platinum levels measured in the spinal cord immediately after cisplatin infusion were not influenced by heat treatment at 42.1 or 43.0°C for 30 min. However, after a heat dose of 60 min 43°C, cisplatin uptake was significantly increased ($P < 0.001$) by a factor of 2.8 (1.3). The data demonstrate that mild hyperthermia has no effect on the uptake of cisplatin in the spinal cord, while an injurious heat dose leads to a significant increase in cisplatin uptake. The present findings indicate that, in case of treatment of tumours of the central nervous system with hyperthermia and cisplatin, a treatment which might be toxic for the tumour is well tolerated by the normal nervous tissue.
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INTRODUCTION

THE MAJOR side-effects of the widely used anticancer drug cisplatin are acute and chronic nephrotoxicity and neuropathy [1–4]. The cisplatin induced nephrotoxicity has however become managable using a regimen of hydration and forced diuresis [5] or by chemoprotection [6]. Neurotoxicity is therefore now considered to be the dose limiting factor in platinum based chemotherapy [7]. The most common neurological complication is peripheral sensory neuropathy, which has been described in detail by a number of clinicians [8–10]. The frequency of neuropathies increased with increasing cumulative doses of cisplatin. Discontinuation of the therapy is generally followed

by recovery, but remaining deficits may be observed. Rare cisplatin related neurological complications include seizures and Lhermitte's sign [11–13]. Lhermitte's sign is a neurological complication characterised by tingling or sensations of electric shock in the arms and/or legs after flexion of the neck due to affection of the spinal cord. The relatively limited central neurotoxicity of cisplatin has been ascribed to the blood–brain barrier (BBB), which prevents significant drug entry into the central nervous system (CNS). Thompson *et al.* [14] demonstrated that platinum levels in peripheral nerves were 10–20 times higher than in the brain and spinal cord of the same patient.

Experimental studies on the effects of hyperthermia on the CNS showed that this tissue is relatively sensitive to heat. Local heat treatment of the brain in cats and dogs indicated a critical upper heat dose at approx. 50–70 min, 42.0–42.5°C [15, 16]. Local heat treatment of the cervical spinal cord of the rat resulted in neurological complications and lethality at heat doses exceeding approx. 60 min, 42°C and 20–30 min, 43°C [17, 18]. Studies on the heat tolerance of the mouse thoracolumbar spinal cord [19] and the rat lumbar spinal cord [20] confirm that nervous tissue may be injured at relatively mild temperatures.

Hyperthermia may alter cisplatin pharmacokinetics, cisplatin uptake in tissue and may potentiate cisplatin toxicity [21–23]. Laboratory studies show reversible interruption of the BBB by hyperthermia, allowing temporary facilitation of delivery of markers to the brains. Heat treatment of rat brain increased for example the uptake of horseradish peroxidase (HRP) after 15 min, 42°C and Evans Blue after 10 min, 43°C [24, 25]. In contrast, Williams *et al.* [26] showed that the BBB permeability for Evans Blue, HRP and fluorescein was decreased after heat treatment for 30–90 min at 40.6–42.7°C. There are no data on the effects of heat on cisplatin passage through the BBB.

Since tumours of the brains and spinal cord generally are radioresistant [27], there is increasing interest in the use of hyperthermia and chemotherapy. Laboratory studies using that combination therapy can provide information on the response of normal tissues and may contribute to prevention of complications in the clinical practice. In the present study, the influence of local hyperthermia on the uptake of cisplatin in the rat cervical spinal cord was investigated. In addition, the possible effect of the combined treatment was assessed on both sensory and motor functions of the animals.

MATERIALS AND METHODS

Animals

Female Wistar: WU (n = 48) and WAG/Rij (n = 32) rats, aged 9–10 weeks and weighing 160–200 g were used in all the experiments (Harlan CPB, Zeist). Animals were anaesthetised with sodium pentobarbital, 50 mg/kg body weight intraperitoneally. In addition, in case of a heat treatment, pentazocine was injected, 1.8 mg/animal intraperitoneally. Animals recovered from anaesthesia in an infant incubator (Air Shields Europe) at 32°C.

Hyperthermia

The cervical region in the rat, including the spinal cord part cervical 5-thoracic 2 was heated using a 434 MHz microwave applicator. Technical aspects of this non-invasive heating system and details on thermometry have been described previously [17]. Briefly, the cervical vertebral column and immediately adjacent tissues could be heated, whereas surrounding tissues where only slightly heated. The temperature was regulated using a reference thermocouple probe, which was placed against one of the cervical vertebrae 6, 7 or thoracic 1. In the experiments, heat treatments were performed for 30, 40 or 60 min at a reference temperature of 42.0 (0.1) °C, 43.0 (0.1) °C or 44.0 (0.1) °C [mean (S.D.)] which resulted in an average maximum temperature in the spinal canal (C5–T2) of, respectively 41.2 (0.4) °C (n = 9), 42.3 (0.4) °C (n = 19) and 43.0 (0.4) °C (n = 27).

Drugs and administration

Cisplatin (Platinol, Bristol Myers) (5 mg/kg body weight) was administered in three different ways. In the first experiment, cisplatin was injected intraperitoneally in a volume of 20 ml of 0.9% NaCl just before hyperthermia was started (Fig. 1:I). A second group of animals was given a single intravenous injection, 20 min after reaching the reference temperature (Fig. 1:II). In the third experiment, cisplatin was administered by intravenous infusion for 2 h, using a Hospal K-10A pump (Hospal Dasco S.p.A.,) at a flow rate of 1.5 ml/h. The spinal cord was heated for 30–60 min. during the infusion period (Fig. 1:IIIA–C).

Follow-up and tissue-sample preparation

Animals with a follow-up of 24 h were inspected and neurological symptoms were scored using an arbitrary graded scoring system [17]. Sensory function was tested by stimulation of the foot sole of both fore- and hindlimbs, as described by De Koning *et al.* [28]. Blood samples were collected by orbita puncture under deep anaesthesia. The samples were centrifuged (Eppendorf 5415 centrifuge, 16 000 g, 3 min) and the plasma was harvested. About 150 µl of the plasma was ultrafiltered through a membrane filter with a 10 kD cut off (Amicon) at 1000 g for 10 min. From all animals, after killing, the cervical and upper thoracic parts of the spinal cord were dissected. Samples were stored at −20°C and platinum concentrations

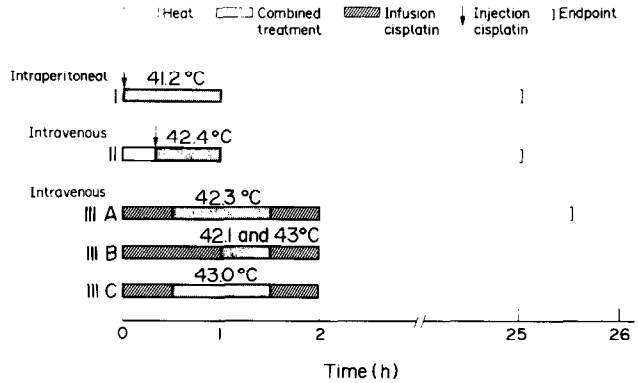

Fig. 1. Experimental set-up of the combined treatmentof cisplatin and hyperthermia on the cervical rat spinal cord. Cisplatin (5 mg/kg body weight) was administered via the intraperitoneal or via the intravenous route by single injection (resp. set-up I and II) or by continuous infusion at a flow rate of 1.5 ml/h (set-up III). The endpoint for tissue sampling was taken either immediately or 24 h after hyperthermia.

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were determined by flameless atomic absorption spectroscopy [29].

Statistics

Significance between the tissue Pt concentrations of the different groups was calculated using the Student's *t* test.

RESULTS

The uptake of cisplatin in the spinal cord after single dose injection combined with local heating

Table 1 lists platinum concentrations measured in different nervous tissues 24 h after single intraperitoneal or intravenous injection of cisplatin with or without local heat treatment of the spinal cord at, respectively 41.2°C for 60 min and 42.4°C for 40 min. (Fig. 1:I and 1:II). At this time, none of the animals expressed either sensory or motor neurological abnormalities. The Table shows, in spite of the fact that the variation in [Pt] was relatively high, no effect of local spinal cord heating on the Pt uptake in different parts of the peripheral and central nervous system. Uptake of cisplatin in peripheral nerves was about 10 times higher than uptake in the spinal cord and brains. Furthermore, no difference was observed in uptake of platinum in the spinal cord between rats of the Wistar and rats of the WAG/Rij strain.

The uptake of cisplatin in the spinal cord after local hyperthermia during continuous infusion

For these experiments, heat was applied for 30–60 min. during a continuous 2 h infusion of cisplatin. Tissues were collected either immediately (Fig. 1:IIIB+C) or 24 h (Fig. 1:IIIA) after treatment. 1 day after hyperthermia for 60 min at 42.3°C, the normal neurological state of animals maintained and platinum concentrations measured in the spinal cord after cisplatin alone and after cisplatin combined with 60 min 42.3°C were not significantly different. Figure 2a shows the platinum content of the spinal cord, kidneys, plasma and ultrafiltrate immediately after cisplatin combined with 30 min 42.1 and 43°C, which is within the safe heat dose range, and after 60 min 43°C, a neurotoxic heat dose. Platinum uptake in the spinal cord was not influenced at the lower heat doses. A significant enhanced uptake of cisplatin in the spinal cord was however measured after 60 min, 43°C. The drug concentration was increased by a factor of 2.8 (1.3). Kidney Pt-levels slightly

Table 1. Platinum concentrations [μg/g tissue, mean (S.E.)] measured in peripheral and central nervous tissues one day after single intraperitoneal or intravenous administration of cisplatin (5 mg/kg) with or without local hyperthermia of the cervical spinal cord (Experimental set-up I and II, n = 2-9)

Treatment	Sham		60 min. 41.2°C	40. min. 42.4°C
Rat strain	WU	WAG/Rij	WU	WAG/Rij
Cervical spinal cord	0.15 (0.04)	0.09 (0.02)	0.14 (0.02)	0.12 (0.02)
Thoraco-lumbar spinal cord	*	*	*	*
Cortex brain	0.06 (0.01)		0.09 (0.01)	
Brachial plexus	1.08 (0.62)		1.39 (0.07)	
Sciatic nerve	0.92 (0.27)		1.17 (0.05)	

* Below detection level of 0.004 μg/g tissue.

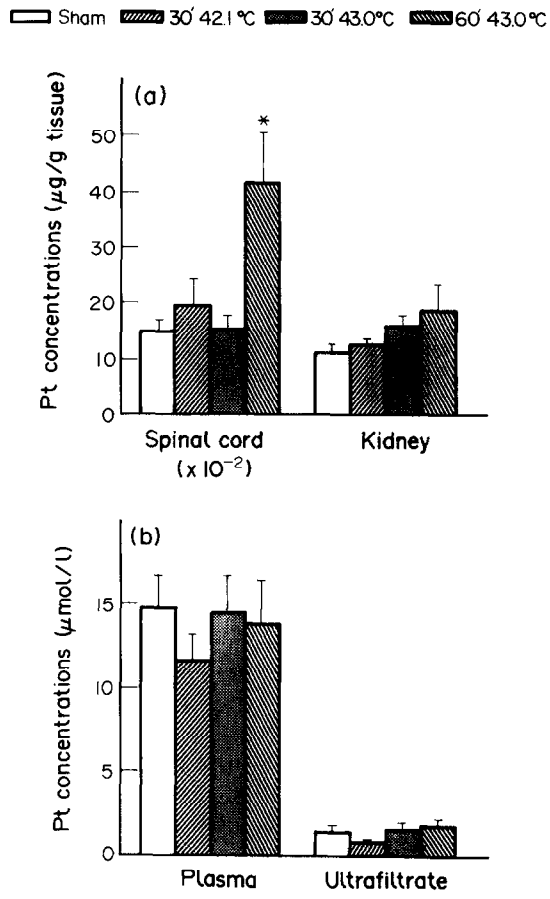

Fig. 2. Platinum content of blood plasma, ultrafiltrate, spinal cord and kidneys after combined treatment of systemic cisplatin and local hyperthermia of the cervical spinal cord of 5–10 animals. Cisplatin was given by infusion at a total dose of 5 mg/kg body weight and hyperthermia was applied to the cervical spinal cord for 30–60 min. at 42.1–43°C. Tissue samples were taken immediately at the end of the infusion period (Fig. 1, set-up IIIB+C) and platinum levels were measured. *$P < 0.001$.

increased from 11.1 (1.6)μg/g tissue to 18.4 (4.7) μg/g tissue (n.s.) with increasing local heat dose on the spinal cord. This is probably the result of a rise of systemic temperature to approximately 40°C at the highest temperature applied locally. The higher platinum uptake in the spinal cord is unquestionably a result of high dose hyperthermia, since both plasma and ultrafiltrate platinum concentrations were almost equal in all treatment groups (Fig. 2b).

DISCUSSION

Neurotoxicity is considered as a serious dose limiting factor in platinum based chemotherapy. Sensory peripheral neuropathy following high cumulative cisplatin dose has been frequently reported [7]. In both animal and human studies, cisplatin treatment resulted in small amplitude action potentials and slower H-reflex related conduction velocities of sensory nerves [30, 31]. These observations are consistent with total platinum concentrations, which are high in spinal ganglia and peripheral nerves, but low in the CNS [14]. Our data confirm that sytemic cisplatin treatment resulted in about 10 times higher platinum levels in the sciatic nerve and in nerves from the brachial plexus than in the brain cortex and the spinal cord of the same animal (cf. Table 1). The lack of cisplatin accumulation in the CNS has been ascribed to the protective effect of the BBB. Disruption

of the BBB may allow drug entrance, leading to neurological complications [32]. Hyperthermia has been shown to affect the BBB and thus might facilitate drug uptake in the CNS. An increase in peripheral neuropathy was observed after local hyperthermia of the rat sciatic nerve combined with systemic cisplatin [33]. They showed an enhancement of heat neurotoxicity after administration of cisplatin in a 6 week regimen. In addition, recovery from heat induced motor neuropathy was delayed in animals that had received cisplatin.

The present study shows that mild hyperthermia of the spinal cord, in the clinical relevant range of 30 min, 42–43°C, does not affect the uptake of cisplatin. No effect of the combined treatment was observed on the neurological state of the animals. Therefore, the heat sensitivity of this vital tissue had not been changed by systemic cisplatin administration. After heating for 1 h at 43°C, a neurotoxic heat dose [18, 34], cisplatin uptake was significantly increased. The data therefore demonstrate that the permeability of the BBB from the normal rat spinal cord for cisplatin was not impaired after mild heat treatment, but only after injurious heat treatment.

The number of clinical studies on the combined treatment of hyperthermia and chemotherapeutic drugs is limited [22]. Laboratory data on the combination of hyperthermia and drugs are scarce but promising. For example using an experimental tumour model in the peritoneal cavity of the rat, it was shown that cisplatin combined with regional hyperthermia for 60 min, at 41.5°C resulted in higher tumour platinum concentrations compared with normal tissues [23]. Long-term prognosis for patients with primary brain- or intraspinal neoplasm is still poor since such tumours generally are radioresistant [27]. Therefore, in the past decade, chemotherapy is being incorporated more and more in the therapy of CNS malignancies. Because of technical limitations and the fear for normal tissue injury, the role of hyperthermia in the treatment of brain and spinal cord neoplasms is still marginal.

In a recent study, Jonson *et al.* [35] showed that platinum uptake in brain tumours of 6 patients was about 10 times higher than in the normal brain tissue. The authors assume that this preferential uptake was caused by violation of the BBB by the malignant tissue. Furthermore, if there are differences in anatomic structure of the BBB between CNS tumours and normal nervous tissue, hyperthermia might facilitate selective delivery of drug to the tumour and, in addition, potentiate chemical killing of malignant cells. The present observations show that in the combined treatment with cisplatin and mild hyperthermia, there is fortunately no increase in toxicity to the normal nervous tissue expected.

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Fatty Acid Composition of Normal and Malignant Cells and Cytotoxicity of Stearic, Oleic and Sterculic Acids *in vitro*

Beverley F. Fermor, John R.W. Masters, Christopher B. Wood, Jayne Miller, Kosta Apostolov and Nagy A. Habib

The aim of this study was to investigate the hypothesis that saturated fatty acids are differentially cytotoxic to cancer cells. Three studies were undertaken to: (1) measure the toxicities of stearic and oleic acids to normal and malignant cells *in vitro*, (2) assess if there is any relationship between toxicity and relative fatty acid composition and (3) determine whether the relative fatty acid composition of a cancer cell line could be modified by sterculic acid, an inhibitor of delta-9-desaturase. Stearic (18:0) and oleic (18:1) acids inhibited the colony-forming abilities of five human cancer cell lines and two non-neoplastic cell lines in a dose-dependent fashion. The concentration of oleic acid required to reduce colony formation ability by 50% was 2.5–6.0-fold greater than that of stearic acid. Addition of sterculic acid to a cancer cell line resulted in steady-state levels of stearic acid and increasing percentage of oleic acid.
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INTRODUCTION

CANCER CELLS appear to have an altered balance of saturated to monounsaturated fatty acids. For example, the ratio of stearic to oleic acid is lower in hepatocellular carcinoma than normal liver [1]. Similarly, increased proportions of oleic acid are found in experimental tumours [2–4], hepatoma cell lines [5] and virally transformed cell lines [6, 7]. These observations led to the concept that tumour cell growth might be modulated by addition of exogenous saturated fatty acids such as stearic acid [8].

The aim of this study was to investigate the possibility that fatty acids possess anticancer activity. Three studies were undertaken. These were: (1) to compare the *in vitro* toxicities of saturated stearic acid with monounsaturated oleic acid to normal and neoplastic cells, (2) to determine whether there is any relationship between percentage fatty acid compositions and toxicity, and (3) to determine if percentage fatty acid composition of one malignant bladder cell line (RT112) is influenced by sterculic acid. This compound inhibits delta-9-desaturase, the enzyme which desaturates stearic to oleic acid [3].

Previous studies *in vitro* have assessed the differential cytotoxicity of stearic and oleic acids to one or two cell lines [9–16]. This is the first study to assess toxicity using a panel of human neoplastic and non-neoplastic cells *in vitro*. Human cancer cell lines were chosen from tumour types with a wide range of sensitivity to chemotherapy, including curable testicular cancer, moderately sensitive bladder cancer and relatively resistant colon cancer. The sensitivities of these cell lines to chemotherapeutic drugs reflects the clinical response of their tumours of origin [17]. The sensitivities of the cancer cell lines were compared with those of a continuous cell line derived from normal urothelium, HU609, and an untransformed normal fibroblast cell line derived from fetal lung, HFL.

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