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SHORT COMMUNICATION

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Distribution of daunorubicin and daunorubicinol in human glioma tumors after administration of liposomal daunorubicin

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Abstract DaunoXome is a liposome formulation containing daunorubicin (DM). Encapsulation of the drug in liposomes presents the advantage of low-level systemic exposure and better drug penetration into the tumor. We studied the distribution of DM and its 13-dihydro metabolite, daunorubicinol (DMol), in surgical biopsies from different parts of glioblastomas. The study was performed in eight patients with recurrent glioblastoma, all of whom had previously undergone surgery and been treated with radiotherapy and chemotherapy, who received 50 mg of DaunoXome as a 1-h infusion. Surgery was performed at 24 and 48 h after the infusion in seven cases and one case, respectively. Biopsies were divided into three parts: the central area of the tumor, peripheral tumor tissue, and brain-adjacent tumor (BAT) tissue. A complete plasma pharmacokinetics study was conducted in seven cases, with samples being taken for up to 48 h after the end of the infusion. DM and DMol were determined in plasma and tissue by high-performance liquid chromatography with fluorescence detection after solvent extraction. At 24 h, concentrations of DM and DMol in the central part of the tumor ranged between < 0.005 and 0.80 μg/g and between 0.005 and 1.58 μg/g, respectively. Concentrations were similar in the peripheral tumor and in BAT tissue. From the data obtained on the patient who underwent surgery at 48 h it appears that DM and DMol remain in tumor tissue for a long time, the concentrations being 0.4 and 2.8 μg/g, respectively. DaunoXome was rapidly cleared from the body, with the plasma levels of DM and DMol determined at 48 h lying in the range of < 5–50 and < 5–20 ng/ml, respectively. The mean ($\pm$SD) half-life and plasmatic clearance of DM were 4.8 ± 1.0 h and 0.2 ± 0.06 l h^{-1} m^{-2}. In conclusion, DaunoXome achieved and maintained potentially cytotoxic levels of both DM and DMol in glioblastoma for a long time in association with low-level systemic exposure. Further studies are therefore warranted. Although only preliminary and obtained in previously treated patients, these data suggest that DaunoXome merits investigation in CNS tumors.

Key words Liposomal daunorubicin · Brain tumor · Tumor level · Pharmacokinetics

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Introduction

Anthracyclines are anticancer drugs that have a broad spectrum of activity but are not effective against brain tumors, possibly because of poor penetration into the central nervous system (CNS) [4]. It therefore seems important that new drug-delivery methods be found to ensure better penetration of drugs that are potentially active against brain tumors.

Recently, with the aim of modifying the pharmacokinetics and the distribution of anthracyclines, drug-carrier liposomes have been developed and investigated in preclinical systems [6, 9, 13]. In Fisher rats, doxorubicin encapsulated in long-circulating liposomes extravasated selectively into a brain tumor, resulting in enhanced tumor drug exposure and greater effect [17] along with much less systemic toxicity than that associated the free drug. Clinical data suggest that liposomes loaded with therapeutic agents have a better therapeutic index than the free drug [7, 15, 18].

DaunoXome consists of daunorubicin encapsulated in liposomes [3]. This combination is stable in the bloodstream and may reach malignant glioma cells by

leaking through the capillary walls of the tumor's new vascular network [12]. In animal models, daunorubicin reached concentrations in tumor tissue that were 10 times higher after liposome-mediated delivery than after delivery of the free drug [5]. In two phase I/II studies in patients with advanced AIDS-related Kaposi's sarcoma (AIDS-KS), Guaglianone et al. [10] and Gill et al. [7] defined the plasma pharmacokinetics of DaunoXome, showing rapid disappearance of daunorubicin.

The limited information available on the drug's pharmacokinetics in patients with CNS malignancies and on tumor tissue levels – represented only by data presented as an abstract [1] – prompted us to perform this study, in which the distribution of daunorubicin and daunorubicinol in glioblastoma tissue and in normal adjacent brain tissue was investigated in a small series of patients who received DaunoXome.

Patients and methods

Patients

This study was reviewed and approved by an ethics committee. Eight patients with recurrent glioblastoma, scheduled for a second debulking, gave informed consent and entered the study. Table 1 reports their main characteristics, including previous and concurrent therapy. All patients were aged less than 65 years and had a Karnofsky performance status (PS) of >70, normal results in complete hematological and biochemistry tests, and normal renal and hepatic function. All had previously undergone surgery and had received both radiotherapy and chemotherapy.

The interval between first and second surgery was at least 8 months, except in cases of multicentric or subependymally disseminated tumors. Each patient received 50 mg of DaunoXome diluted with 5% dextrose solution to obtain a concentration of 1 mg/ml and given as a 1 h i.v. infusion. Surgery was done at 24 and 48 h after the end of the infusion in seven cases and in one case, respectively. The excised tumor was divided into three parts – the central tumor, peripheral tumor (peritumoral) tissue, and brain-adjacent tumor (BAT) tissue – for study of the drug's distribution in different areas.

A plasma sample was taken from all patients at the time of tumor dissection and a complete pharmacokinetics study was done in seven patients, with plasma being collected at the following times: before the infusion, at the end of the infusion, and at 0.5, 1, 3, 6, 12, 24, and 48 h after the infusion. Tissue and plasma samples were stored frozen at −20 °C until analysis.

Drug assay and pharmacokinetic calculations

Daunorubicin (DM) and daunorubicinol (Dmol) were determined in plasma and tissue by high-performance liquid chromatography (HPLC) with fluorimetric detection according to a previously described technique [2], with minor modifications. In brief, 0.5–1 ml of plasma or 1 ml of tissue homogenized in water was added to 1 μg of doxorubicin as the internal standard and 1 ml of borate buffer (pH 8.4), deproteinized with $AgNO_3$ (33%), extracted with 8 ml of chloroform, and centrifuged at 3500 rpm. The organic phase was evaporated under vacuum. Extracts were dissolved in 0.1 *M* phosphoric acid and 50 μl was injected into the HPLC system 501 module (Waters, Milford, USA) equipped with a fluorescence detector (model 474, Waters) operating at an excitation wavelength of 475 nm and an emission wavelength of 580 nm. Separation was achieved with an isocratic solvent system of acetonitrile:water:0.1 *M* phosphoric acid (pH 2.7; 30/44/26, by vol) at a flow rate of 1.5 ml/min using a μBondapak C18 column, (4.6 × 30 mm, Waters).

The retention times of doxorubicin, Dmol, and DM were 5.6, 6.5, and 10.5 min, respectively. The limit of detection was 5 ng/g for tissue and 5 ng/ml for plasma. To determine the main pharmacokinetic parameters of DaunoXome, we elaborated the concentration-time data points of DM and Dmol according to a general nonlinear fitting program [16].

Results

Complete plasma pharmacokinetic profiles were obtained for seven patients. The mean plasma-decay curves generated for DM and DMol in patients receiving DaunoXome are shown in Fig. 1. After the end of the infusion, DM plasma levels declined rapidly, following a one-compartmental model and showing fast elimination and little interpatient variability. Peak plasma concentrations (C_{max}) ranged from 4.8 to 21.8 μg/ml, and the mean (±SD) half-life, plasma clearance, and volume of distribution were 4.8 ± 1.0 h [coefficient of variation (CV) 27%], 0.20 ± 0.06 $l\,h^{-1}\,m^{-2}$ (CV 30%), and 1.35 ± 0.17 l/m^2 (CV 12%). Plasma levels of DMol were low, the 48-h AUC for this metabolite being less than 5% of the AUC of DM. In most cases, plasma concentrations were lower than tissue concentrations at tumor dissection, and at 24 h they were 0.040–0.972 and <0.005–0.16 μg/ml for DM and DMol, respectively.

The DM and Dmol concentrations detected in the central tumor, peritumoral tissue, BAT tissue, and plasma of patients are reported in Table 2. In six of the seven patients given DaunoXome at 24 h before surgery the concentrations in the tumor ranged from 0.005 to 0.803 μg/g (median 0.056 μg/g) for DM and from 0.014 to 1.581 μg/g (0.125 μg/g) for the metabolite; comparable levels were found in the peritumoral tissue and in BAT tissue. In one case no anthracycline was found in tumor

Table 1 Characteristics of the patients (*GBM* Glioblastoma multiforme, *KPS* Karnofsky performance status, *CDDP* cisplatin, *BCNU* carmustine, *RT* radiotherapy)

Initials	Age (years)	Site of lesion and histology	KPS	Previous treatment	Body surface (m^2)
FR	41	Left temporal GBM	100	CDDP + BCNU + 58 Gy RT	1.65
VA	52	Right frontal GBM	90	CDDP + BCNU + 57 Gy RT	2.00
PS	35	Left parietal GBM	90	CDDP + BCNU + 60 Gy RT	1.60
CA	39	Left parietal GBM	80	CDDP + BCNU + 54 Gy RT	1.70
CR	51	Right occipital GBM	90	CDDP + BCNU + 60 Gy RT	1.90
FM	63	Right frontal GBM	80	CDDP + BCNU + 57 Gy RT	1.75
PD	59	Left frontal GBM	90	CDDP + BCNU + 60Gy RT	1.85
TA	49	Right temporal GBM	90	CDDP + BCNU + 60 Gy RT	1.75

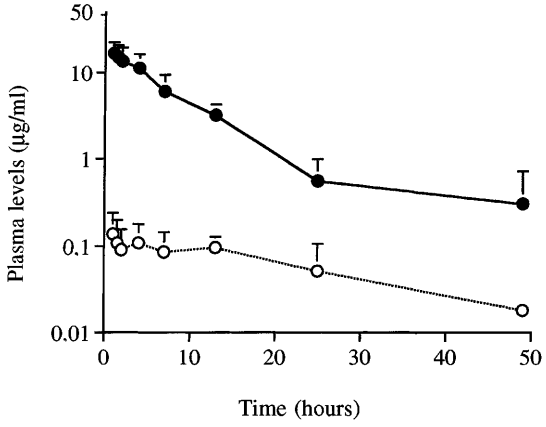

Fig. 1 Mean (±SD) plasma levels of DM (●) and DMol (○) determined in 7 patients who received 50 mg of DaunoXome as a 1-h infusion

biopsies (< 5 ng/g). In the patient given DaunoXome at 48 h before surgery, tissue concentrations of Dm and Dmol were higher than the median values observed at 24 h in the other patients, being 0.40, 0.43, and 0.168 μg/g and 2.819, 1.350, and 1.150 μg/g in the central tumor, peritumoral tissue and BAT tissue, respectively.

Discussion

Limited drug distribution is the main obstacle in CNS malignancies because most agents do not cross the blood-brain barrier efficiently [4]. Anthracyclines are currently employed for the treatment of several tumors but are not used in brain tumors because of their limited ability to cross the blood-brain barrier. To our knowledge, only one report has been published on the penetration of anthracyclines in human glioma. Von Holst et al. [19] found doxorubicin tumor levels ranging between 0.9 and 4.6 μg/g in patients receiving 50 mg of doxorubicin, but the drug was not detectable in the surrounding tissue, where infiltrating tumor cells are generally present.

Previous studies have indicated that the liposomal formulation gives better drug delivery into the tumor [6, 9, 13]. Doxorubicin or DM encapsulated in liposomes were more efficacious than the free drugs in animal tumor models [5, 17]. DaunoXome showed activity in patients with Kaposi's sarcoma [8], the considerable advantage being that the liposomal encapsulation gave a low toxicity profile due to the low-level systemic exposure to free DM [7].

In previous papers [10, 15] the plasma pharmacokinetics of DaunoXome was described by a monoexponential decline associated with a short half-life and considerably lower clearance than that of free DM, but little information was provided about Dmol, which presumably reaches only a very low concentration. Our study confirms the rapid disappearance of the drug from plasma with monoexponential kinetics, also providing data on the amount of the metabolite. The low-level of systemic exposure to the metabolite was in line with the observation that the treatment appeared safe and well tolerated in these patients.

In seven of eight patients we found considerable levels of both DM and DMol in the central tumor tissue, peritumoral tissue, and BAT tissues, confirming the observation by Albrecht et al. [1]. These concentrations do not appear to be related to the plasma levels measured at the same time. In tissues the metabolite reached levels that were 1.5–5 times higher than those recorded for DM despite the finding that the plasma DMol AUC was 20 times lower than the AUC of the parent compound.

The concentration at which DM is effective in glioma tumors is not known, and there is no detailed information on its activity in glioma cells. Recently it was reported that organotypic multicellular spheroids prepared from seven glioma specimens were highly sensitive to a 2-μg/ml concentration of Dm or doxorubicin [11, 14], but not to carmustine, which is considered the most effective agent for glioma. According to these data the concentrations of the parent drug and the active metabolite found in brain neoplasms and in the BAT tissues – where infiltrating cancer cells are likely to be present – are on the same order of magnitude as the

Table 2 DM and DMol concentrations detected in the central tumor, peritumoral tissue, BAT tissue, and plasma of patients at 24 h after the administration of 50 mg of DaunoXome (*NA* Sample not available)

Initials	Central tumor		Peritumoral tissue		BAT		Plasma	
	DM (μg/g)	DMol	DM (μg/g)	DMol	DM (μg/g)	DMol	DM (μg/ml)	DMol
FR	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.900	0.160
VA	0.060	0.125	0.141	0.372	< 0.005	< 0.005	0.040	0.022
PS	0.803	1.390	NA	NA	NA	NA	0.32	< 0.005
CA	0.345	1.581	0.432	1.887	0.483	1.105	0.972	0.043
FM	0.043	0.224	0.028	0.047	0.047	0.145	0.011	0.012
PD	0.010	0.014	0.014	0.015	0.018	0.021	1.013	0.051
TA	0.056	0.094	0.040	0.047	0.095	0.250	0.65	0.023
CR[a]	0.400	2.819	0.430	1.350	0.168	1.150	0.045	0.050

[a] For this patient, all samples were taken at 48 h

concentrations that are cytotoxic in vitro, but this extrapolation must be made with caution, considering the possible differences between the in vitro and in vivo situations.

In conclusion, DaunoXome achieves potentially cytotoxic drug concentrations in human gliomas in association with low-levels of systemic exposure and toxicity. These pharmacological data provide a rationale for clinical studies aimed at assessing the potential activity of this liposomal preparation of DM for CNS tumors.

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