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Uptake of Adriamycin in Tumour and Surrounding Brain Tissue in Patients with Malignant Gliomas

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Summary

Eight patients with malignant gliomas verified on CT scan, received an intravenous injection of 50 mg of Adriamycin R, 24 hours prior to surgical removal of the tumour. Peroperatively, both tumour and surrounding tissue specimens were obtained for determination of the tissue concentrations of Adriamycin and its reduced metabolite Adriamycinol. It was found that Adriamycin could be detected in tumour tissue from all patients. The concentration varied between 0,9 and 4,6 nmol/g tissue. In contrast, Adriamycin could only be detected in surrounding brain tissue from one patient.

In an *in vitro* study a human malignant glioma cell line (U-251 MG) was exposed to various concentrations of Adriamycin for 24 hours. It was found that an intracellular drug concentration above 30 nmol/g cells caused a concentration dependent inhibition of cell growth. Thus, it is likely that the poor effect of Adriamycin on patients with malignant gliomas is due to an ineffective drug accumulation in the tumour tissue.

Keywords: Adriamycin; malignant gliomas; glioblastoma; chemotherapy; tissue concentration.

Introduction

Despite surgery and aggressive radiation therapy, survival of patients with malignant gliomas is extremely poor with practically no five-year survival[1]. The clinical value of chemotherapy is also debated. So far many drugs have been tried but with little therapeutic success. Nitrosoureas have produced the highest response rates when used as single agents[1].

Doxorubicin is an antracycline antibiotic which acts by intercalation in the DNA[2]. It has a broad antitumour spectrum with effect on a large number of haematologic and solid malignancies. No convincing effect has been found on malignant gliomas. Patients with solid tumours metastasizing to the CNS may respond systemically while the CNS metastases progress[3]. Intracarotid infusion of Adriamycin in combination with Mannitol in order to open the blood brain barrier at the tumour site of glioblastomas has shown no significant improvement in survival time[4]. Furthermore, trials with intracavitary Adriamycin have been performed through an Ommaya reservoir but with very limited clinical effect[5]. One possibility for the poor clinical outcome following Adriamycin treatment could be that the drug concentration in the tumour does not reach cytotoxic levels.

Therefore, the aim of the present study was:

– to determine the concentration of Adriamycin in human malignant gliomas and surrounding tissue after intravenous drug administration prior to surgery and

– to determine the concentration – effect relationship for the cytotoxicity of Adriamycin in human malignant glioma cells *in vitro*.

Materials and Methods

Patients

About 24 hours before surgery, 50 mg of Adriamycin R (Farmitalia Carlo Erba) was injected intravenously into 8 patients suffering from malignant gliomas during the course of 10 minutes and followed by a 10 ml saline injection. No side effects were reported.

During neurosurgical removal of the glioblastomas, tumour specimens were obtained from the central necrotic area and the peripheral viable tumour tissue. Cerebral tissue surrounding the tumour was also studied. Control pieces of each tissue specimen for doxorubicin analysis were taken for histopathological evaluation and the remainder of each tissue piece was frozen and stored at −20 °C until the concentration of doxorubicin was analysed.

Drug Analysis

Plasma concentrations of Adriamycin and its reduced metabolite Adriamycinol were analysed by high pressure liquid chromatography

(HPLC) as previously described[6]. In brief, a 0,2 ml aliquot was added to 0,2 ml of 0,1 M borate buffer (pH 9,8) containing daunorubicin as internal standard. The drugs were extracted with 1,8 ml of chloroform/methanol (4 : 1 by volume). Separation was performed on a Lichrosorb Si-60 column (Hibar, 25 cm × 4 mm, 7 µm, from E. Merck, Darmstadt, FRG) eluted with a mixture of chloroform, methanol, glacial acetic acid, and 0,3 mM MgCl2 (720 : 210 : 40 : 30 by volume) at a flow rate of 1,5 ml/minute. The column outlet was connected to a Gilson model FL-B fluorometer (Gilson Medical Electronics Inc, Middleton, WI) and the fluorescence signal integrated by a Chromotapac data processor (Shimadzu Seisakushu Ltd, Kyoto, Japan). After thawing the tissue specimens, 2 ml of 0,1 M phosphate buffer (pH 8,1), containing 1 nmol of daunorubicin as an internal standard were added to 0,5–1 g of tissue. The tissue samples were homogenized with two 5-sec pulses using a Kinematica polytron at setting 5, followed by sonification for 30 seconds. Extraction, separation, and analysis were then performed as for plasma samples.

Cells

Human malignant glioma cells (U-251 MG) were grown in monolayer culture in a medium consisting of RPMI 1640 with 10% fetal calf serum, 1% L-glutamine and antibiotics (100 IU penicillin + 100 µg streptomycin per ml medium). Before each experiment, confluent cells in stock flasks (175 cm^2) were detached by treatment with 0,05% trypsin and 0,02% EDTA and seeded in petri dishes (60 cm^2). After incubation overnight, the medium was changed to fresh medium containing Adriamycin. After incubation for 24 hours, certain dishes were used for the determination of drug uptake. These dishes were washed 3 times with PBS. One ml of PBS was then added and the cells detached with a rubber policeman. This procedure was repeated once. After sonification, Adriamycin was determined as previously described and cell protein was determined by the Lowry assay[7]. In other dishes, the medium was replaced by fresh drug-free medium and the cells were reincubated for an additional 72 hours. The cells were then washed and detached by the addition of 2 ml 0,1 M NaOH before the determination of total protein per dish. Cell growth was determined as the increase in total protein during the incubation.

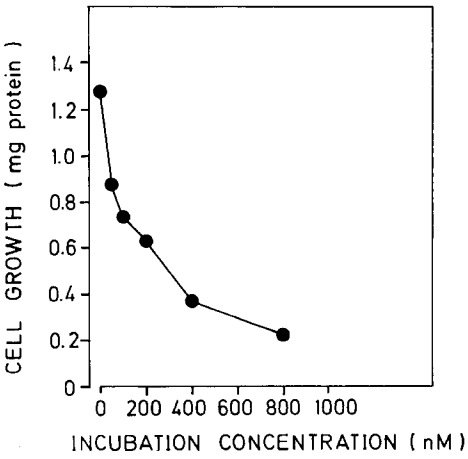

Fig. 1. Effect of Adriamycin on U-251 MG cell growth. The cells were subcultured on day 0 and on day 1 incubated with Adramycin for 24 hours. Thereafter the cells were washed and reincubated in a drug-free medium for another 72 hours. Cell protein was then determined

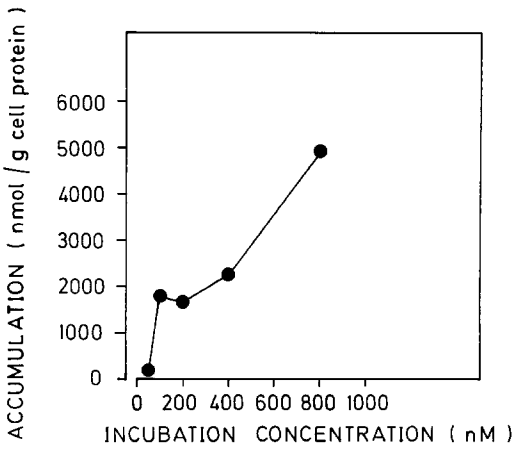

Fig. 2. Cellular drug accumulation U-251 MG cells following 24 hours of drug exposure

Results

In all patients, Adriamycin could be detected in tumour tissue obtained 24 hours after an iv injection of 50 mg drug (Table 1). The plasma concentrations of Adriamycin and its reduced metabolite Adriamycinol were determined at the time of surgery in two of the patients. The concentrations of both parent drug and

Table 1

Patient	Sex/age	Tumour		Surrounding normal tissue	
		nmol adr/g	nmol adrol/g	nmol adr/g	nmol adrol/g
1	m/53	2.28	n.d.	n.d.	n.d.
2	m/60	0.89	n.d.	n.d.	n.d.
3	m/43	0.94	n.d.	–	–
4	f/55	1.35	n.d.	–	–
5	m/46	1.10	n.d.	n.d.	n.d.
6	m/40	1.14	0.62	–	–
7	m/45	4.60	2.00	0.44	0.24
8	m/73	1.05	0.14	n.d.	0.08

n.d. = not detected.

metabolite were about 20 nM (not shown). Since the tissue concentration of Adriamycin was in the order of 1 nmol/g tissue, it exceeded the plasma concentration by a factor of 50.

A sample of necrotic tissue was also obtained from some patients. In all of these cases, less drug was found in necrotic tissue as compared to viable tumour tissue (not shown).

Samples of surrounding brain tissue were also obtained from 7 patients. However histological examination revealed that the macroscopically normal brain tissue was infiltrated by tumour cells in two of these patients. Thus, specimens from 5 patients were histologically considered normal or containing reactive gliosis, but no tumour infiltration. In only one of these (patient No. 7), detectable amounts of Adriamycin could be found in the normal brain tissue.

In vitro Studies

In an attempt to evaluate the cytotoxic potential of the Adriamycin concentrations achieved in the tumour tissue, a human malignant glioma cell line was exposed to various concentrations of Adriamycin for 24 hours and then washed and reincubated in a drug free medium. It was found that Adriamycin (50–800 nM) caused a concentration dependent inhibition of cell growth (Fig. 1). At the end of the incubation with Adriamycin, the cellular drug concentration amounted to 160–4,900 nmol/g cell protein (Fig. 2). Assuming that protein constitutes about 20% of the cellular wet weight, the drug concentration corresponds to 32–980 nmol/g cells. About 350 nmol/g cells caused a 50% growth inhibition of malignant glioma cells. This is between 70–380 times higher than the drug concentration found in tumour tissue from the patients.

Discussion

It is generally believed that the reason for the poor effects of cancer chemotherapy in the treatment of brain tumours is an ineffective drug supply due to the blood-brain barrier. This opinion is supported by results indicating that lipid-soluble drugs like nitrosoureas are most effective. It is also supported by observations that chemotherapy is seldom effective in the treatment of brain metastases even if a clear effect can be seen on the tumour elsewhere[3]. However, this study shows that Adriamycin accumulates to a much higher extent in glioma tissue than in surrounding brain tissue. The tumour tissue concentration of Adriamycin is, however, far below the cytotoxic level for the *in vitro* cultivated cells.

Sakai and coworkers estimated the Adriamycin concentration in brain tumour tissue after intraoperative administration (20 mg by intracarotid injection)[8]. Total fluorescence assay showed concentrations corresponding to 6,5–11 nmol/g tumour tissue. Surrounding brain tissue was not studied. Adriamycin concentrations have previously been determined in gastrointestinal tumours after a peroperative injection of 10 mg drug[9]. The tumour drug concentrations were in the same range as in the present study. From experimental studies it is known that one mechanism of resistance to anthracyclines is an enhanced drug efflux due to the occurrence of a p-glycoprotein in the cell membrane[10]. Expression of the mdrl gene in certain normal tissues like colon and kidney is probably the reason for the poor effect of anthracyclines in the treatment of tumours in these organs. No information is available on the occurrence of p-glycoprotein in malignant glioma tissue. However the present results support the opinion that low drug accumulation in the malignant glioma tissue is responsible for the poor clinical effect of Adriamycin in this tumour type. If the drug accumulation in the tumour could be substantially increased, a therapeutic effect could be expected.

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