Bleomycin treatment of brain tumors: an evaluation

Mette Linnert and Julie Gehl

Bleomycin has been used in the treatment of brain tumors for over 30 years. Currently, we are evaluating electrochemotherapy (the use of electric pulses to enhance uptake of bleomycin) for patients with secondary brain tumors. We, therefore, reviewed the literature with specific reference to the tolerability and toxicity of bleomycin. Using the keywords 'brain' and 'bleomycin', a database search without date restriction was performed and over 500 articles were found. Twenty-five articles were used for this study based on relevance determined by: (i) clinical studies, (ii) use of bleomycin, and (iii) direct injection into brain tissue or cysts. There were two main indications for the use of bleomycin directly into the brain: (i) cystic tumors in the form of craniopharyngiomas and (ii) solid brain tumors such as glioblastomas and astrocytomas. The most frequent adverse effects reported were transient fever, headaches, nausea and vomiting, lethargy, and peritumoral edema. Out of 189 patients treated from 1973 to 2007, only five patients (3%) had severe and six patients (3%) had moderate adverse effects. One death was directly related to this treatment, where very high doses were used. Two patients developed loss of vision and two patients had hearing loss because of the treatment. All cases with severe and moderate adverse effects except one were patients with craniopharyngiomas and probably because of tumor localization in the deep brain. In conclusion, bleomycin injection into the brain has been fairly well tolerated at doses much higher than that used in electrochemotherapy. *Anti-Cancer Drugs* 20:157–164 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2009, **20**:157–164

Keywords: adverse effects, bleomycin, brain, clinical trial, craniopharyngioma, electrochemotherapy, electroporation, intracerebral, intracystic, intratumoral

Department of Oncology, Copenhagen University Hospital Herlev, Herlev, Denmark

Correspondence to Julie Gehl, MD, Department of Oncology, 54B1, Copenhagen University Hospital Herlev, Herlev Ringvej 75, Herlev 2730, Denmark
Tel: +45 44 88 44 88; fax: +45 44 88 34 71; e-mail: juge@heh.regionh.dk

Received 29 September 2008 **Revised form accepted** 5 December 2008

Introduction

Intratumoral bleomycin has been used in the treatment of brain tumors at least since 1973 [1]. The rationale for the use of bleomycin directly into brain tumors is to avoid the problem of passage through the blood–brain barrier (BBB). In general, the BBB is nonpermeable to many types of chemotherapy, and especially a large hydrophilic molecule such as bleomycin [2]. It makes sense to circumvent the BBB by giving chemotherapy directly into the brain tumors or brain cysts, because the dose delivered to the tumor can get much higher than with systemic treatment. At the same time, systemic adverse effects can be minimized. In the literature, there are two main indications for the use of bleomycin directly into the brain: (i) cystic brain tumors in the form of craniopharyngiomas and (ii) solid brain tumors such as glioblastomas and astrocytomas.

In the treatment of craniopharyngiomas, this approach has been used because of several factors such as location of tumor; the age of patients; and the problem of recurrence despite extensive surgery and radiotherapy [3]. Craniopharyngiomas exist in three different morphological types, the majority has a cystic component and are mixed solid/cystic, some are only cystic and about 10% are completely solid. It is the cystic part of the tumors that responds well to intracystic bleomycin, whereas the solid part is often removed by surgery before bleomycin therapy [3–5]. The cystic part is very difficult to completely remove by surgery and has, therefore, a great tendency to recur [4,6]. Intracystic bleomycin has been an overall tolerable and effective treatment of craniopharyngiomas when the tumor is inoperable, recurrent, or in the situation where the age of the patient calls for a treatment that can postpone more drastic and risky measures such as surgery or radiotherapy [3,4,7–9].

In the treatment of solid brain tumors, bleomycin has been used because of some effect against astrocytic tumors in animal models and clinical trials [1,2] and also in an attempt to find an effective treatment for recurrence of solid brain tumors. Results have not been very encouraging, probably because bleomycin does not enter the cells easily. However, it was concluded from animal studies that a small dose of intratumoral bleomycin was more effective than a 25 times larger parenteral dose of bleomycin [2] and this certainly confirms the theory that bypassing the BBB gives better treatment results.

We wanted to review the literature about the use of bleomycin in the brain, because we find that an even better way to pass the BBB is to use electroporation of the tumor cells, that is, electrochemotherapy. In 1993, Salford *et al.* [10] performed electrochemotherapy with bleomycin on rats with inoculated brain tumors with

0959-4973 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328325465e

encouraging results; the survival time almost doubled that of untreated animals. Our goal was to determine the tolerability of treatment with intracerebral bleomycin is in the clinical setting and to examine the nature and extent of toxicity. This is valuable information for later use of electrochemotherapy with bleomycin in the treatment of primary and secondary brain tumors.

Methods

We performed a database search for articles in Medline (*www.ncbi.ntm.nih.gov*) and ISI – Web of knowledge (*www.apps.isiknowledge.com*) with the keywords 'brain' and 'bleomycin' without date restriction. A cross-check of reference lists of the identified articles was also performed. In this way, we found more than 500 articles, and 25 articles have been used for this study based on relevance determined by:

(1) clinical studies,
(2) use of bleomycin,
(3) direct injection into the brain tissue or brain cysts.

Study population

Overall, 189 patients were treated with intratumoral or intracystic bleomycin in brain tumors of the following histologies: 134 (71%) patients with craniopharyngiomas (benign stratified epithelium), 47 (25%) patients with glioblastomas (WHO grades 3 and 4), two (1%) patients with low-grade astrocytomas (WHO grades 1 and 2), two (1%) patients with ependymoma and sarcoma, respectively, and one patient with malignant oligodendroglioma and one patient with medulloblastoma. Most studies are quite small with numbers of patients varying from three to 25 patients or case reports (Table 1).

Table 1 Studies of intratumoral and intracystic use of bleomycin in solid and cystic brain tumors listed by publication year

Studies	Diagnosis	Patients	Age	Bleomycin	Radiotherapy	Adverse effects
[11]	Glioblastoma	3	Not reported	15–45 mg (5 mg); 27–80 U (9 U) every other day	Yes, some	Yes, 1 moderate
[1]	Diverse brain tumors	12	5–57 years	30–80 mg (0.1–0.2 mg/kg); 17–348 U (0.18–0.36 U/kg) every other day	Coirradiation, 5–6000 rad	Yes, mild
[2]	Diverse brain tumors	8	26–68 years	30–225 U (2.5–10 U) daily	Yes, 5–6000 rad WBRT	Yes, mild
[12]	Craniopharyngioma	7	2–13 years	13–95 mg (1–5 mg); 23–170 U (1.8–9 U) every other day	Yes, some	Yes, mild
[7]	Craniopharyngioma	18	4–65 years	5–55 mg (5 mg); 9–98 U (9 U) every other day	No	Yes, 1 severe (deaf) 1Q
[13]	Craniopharyngioma	1	48 years	24.5 mg (0.5–3 mg); 44 U (0.9–5 U) 8 weekly, 4 every other week	No	Yes, 1 moderate
[14]	Craniopharyngioma	6	20 months to 65 years	Not reported	Not reported	Yes, 1 severe (deaf)
[15]	Craniopharyngioma	1	16 years	80 mg (10 mg); 143 U (18 U) in 8 days	No	None
[16]	Craniopharyngioma	1	34 years	5 mg (9 U) one time	Yes	Yes, 1Q
[17]	Craniopharyngioma	1	11 years	220 mg (10–17.5 mg); 393 U (17.8–31 U) daily	No	None
[18]	Craniopharyngioma	1	47 years	56 mg (7 mg); 100 U (12.5) in 8 days	No	Yes, 1 severe (fatal)
[8]	Glioblastoma	25	Mean 55 years	16 mg (1 mg); 29 U (1,8 U) day 1 + 2 in 20-day cycles	60 Gy	Yes, mild
[19]	Astrocytoma	1	6 years	15 mg (3 mg); 27 U (5.4 U) in 5 days	Proton 5400 + boost 900 Cobalt cGy	None
[3]	Craniopharyngioma	9	7–12 years	18–115 mg (2–5 mg); 32–205 U (3.6–9 U) 3 times a week for 3–5 weeks	No	Yes, 1 moderate
[4]	Craniopharyngioma	24	3 months to 64 years	28–150 mg (3 mg); 50–268 U (5 U) every other day	No	Yes, 1 severe (blind)
[20]	Glioblastoma	9	45–65 years	(5–34 U/week continuous) median cumulative dose 195 U	$\geq$ 55 Gy	Yes, mild
[21]	Craniopharyngioma	10	3–67 years	15–180 mg (2–5 mg); 27–321 U (3.7–9 U) every 2–7 days	No	Yes, 1Q
[22]	Craniopharyngioma	1	19 years	45 mg (7.5 mg); 80 U (13 U) and 30 mg (5 mg); 56 U (9 U) in 12 days = 75 mg (140 U)	Yes	Yes, mild
[23]	Craniopharyngioma	5	2–58 years	60–110 mg (5–15 mg); 107–196 U (9–27 U) daily in 8 days	No	Yes, 1Q
[24]	Craniopharyngioma	8	12–74 years	3–35 mg (1.5–3 mg); 5.4–62.5 U (2.7–5.4 U) weekly	No	None
[6]	Craniopharyngioma	11	2–14 years	Minimum of 40 mg (1–5 mg); (1.8–9 U) every other day	Yes	Yes, mild
[5][a]	Craniopharyngioma	11[a]	3–67 years	15–180 mg (2–5 mg); 27–321 (3.7–9 U) per 2–7 days	No	Yes, mild
[25]	Craniopharyngioma	8	5–12 years	Minimum of 33 mg (3 mg); minimum of 60 U (5.4 U) 3 times a week	No	Yes, mild
[26]	Craniopharyngioma	1	14 years	75 mg (5 mg); 141 U (9 U) over 5 weeks	Gammaknife 24 Gy	Yes, severe (blind, one eye)
[9]	Craniopharyngioma	17	1–14 years	8–75 mg (2–15 mg); 14–134 U (3.6–9 U) 1–3 times a week	Maybe some	Yes, 3 moderate
Total		189				5 severe, 6 moderate, 4Q

Values within the brackets represent single dose per treatment.
Q, questionable adverse effect; WBRT, whole-brain radiation therapy.
[a]Ten of 11 patients previously reported by Park *et al* [21].

Dose of bleomycin

The potency of bleomycin is measured in units of microbial activity with 1 U containing 0.56–0.66 mg of bleomycin. One unit is equivalent to 1000 IU [27,28]. This study is based on 1 U containing 0.56 mg of bleomycin.

The doses and the treatment regimes used are very variable (Table 1). The range of single doses for craniopharyngiomas is 5–17.5 mg (2.7–31 U) of bleomycin and for solid brain tumors 1–5 mg (1.8–10 U) of bleomycin, not taking Patchell *et al.*'s [20] dose of 34 U/week into account, because it was administered continuously. There seems to be an agreement to use less than 5 mg (9 U) of bleomycin per treatment (65% of the studies). The frequency and intervals between treatments also differ and, therefore, a wide span of maximum doses was used as outlined in Fig. 1. The frequency varies from daily injections to twice every 3 weeks. The adverse effects related to the maximum dose of bleomycin used in solid tumors are outlined in Table 2.

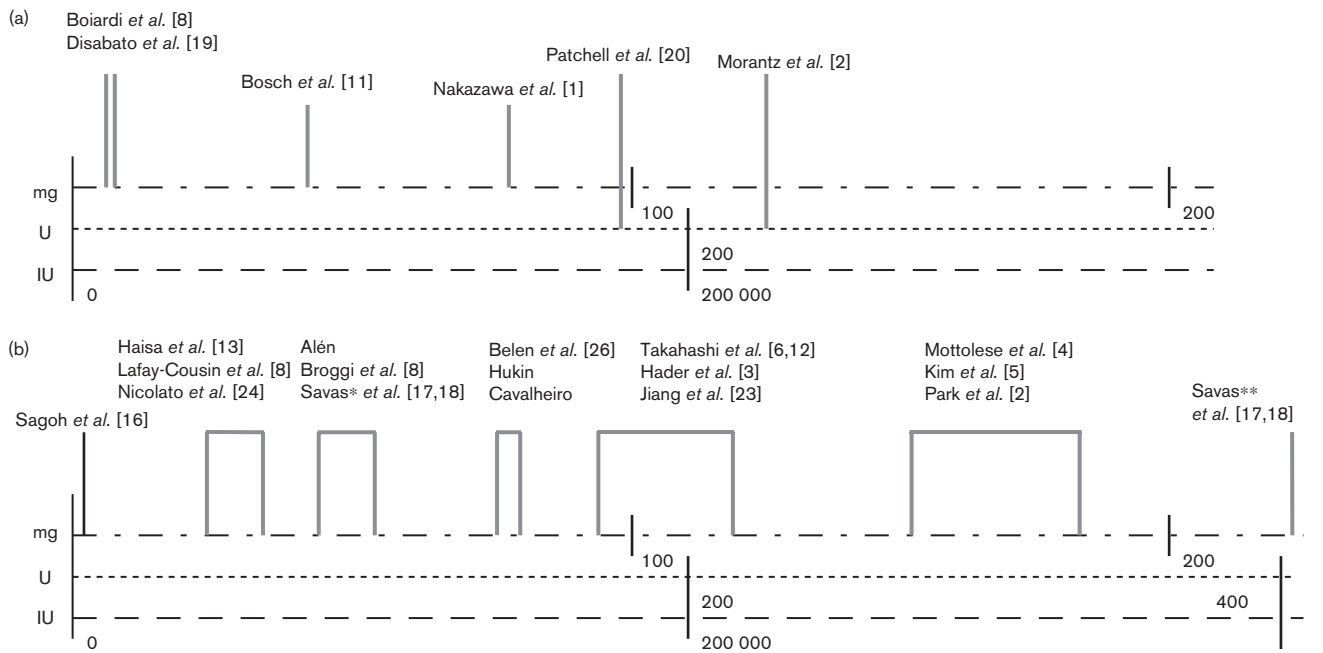
In a dose-escalating study, Patchell *et al.* [20] established a tolerable dose of bleomycin to be 16 U/week when given intratumorally and continuously to patients with recurrent glioblastoma multiforme. Patchell *et al.* [20] also found that the most common toxicity in the study was headache (44%), which resolved when doses were lowered to 16 U/week. Actually, all neurotoxicity resolved when they decreased the dose and added corticosteroid therapy [20]. Lafay-Cousin *et al.* [25] also found a tolerable cumulative dose of 33 mg (62 U) before any adverse effects occurred. Morantz *et al.* [2] concluded that a single dose of 7.5 U could be safely administered because the only patient with any adverse effects had received 10 U per dose. Hukin *et al.* [9] found that a dose larger than 27 U/week or 1.6 U/kg/week was associated with greater toxicity.

Table 2 Studies of maximal cumulative doses of bleomycin used related to adverse effects in solid brain tumors

Studies	Patients	Maximum dose	Adverse effects
[8]	25	16 mg (29 U)	Headache and slight lethargy 4–6 h, vomiting and epilepsy (three patients, antiepileptic medicine below therapeutic range)
[20]	9	195 U (109 mg)	Headache and lethargy
[1]	8	80 mg (348 U)	Transient mild fever, headaches, and nausea
[2]	8	225 U (126 mg)	Headache and lethargy caused by peritumoral edema (CT). Resolved with corticosteroids
[11]	3	45 mg (80 U)	Raised ICP and emergency surgery, worsened hemiplegia, and difficulties in speaking
[19]	1	15 mg (25 U)	No complications

Values within the brackets are converted to milligrams or units depending on which unit was given in the study.
CT, computed tomography; ICP, intracranial pressure.

Fig. 1



Scales of maximum doses of bleomycin used in studies of solid (a) and cystic (b) brain tumors. *One case/no adverse effects. **One case/fatal adverse effects .

Method of delivery

The delivery of bleomycin to the tumor site was in most studies (68%) achieved by implanting an Ommaya reservoir or using a similar method. The reservoir is placed under the scalp with the catheter tip positioned in the brain tumor or cyst that needs to be treated [4]. By implanting the Ommaya reservoir, multiple administrations of chemotherapy may be given through a single access site, thereby lowering the risk of infection and reducing the stress and pain associated with repeated intracerebral injections. Patchell *et al.* [20] modified the reservoir to obtain sustained release of the drug rather than the conventional bolus dose.

The volume of each injection varies between 0.5 and 6 cm^3 when injecting craniopharyngiomas, if the large injection volumes used by Hukin *et al.* [9] (i.e. 2.5–296 cm^3) are not included. In the treatment of craniopharyngiomas, cystic fluid was usually withdrawn before injecting bleomycin.

The injection volume used in the treatment of solid tumors was 0.5–5 cm^3; though the 5 cm^3 bleomycin was inserted over 5 h, because the drug must diffuse through the extracellular space down the concentration gradient [11]. Thus, injection of fluid into solid brain tissue is an entirely different matter than installing chemotherapy into a brain cyst.

Adverse effects

The reports of adverse effects were very variable, with some studies very detailed in this area and others failing to mention the subject at all. A certain report bias is to be expected given that the focus of the studies differs.

Transient fever

The most frequently reported adverse effect, with reports from at least 36 (19%) patients, was transient elevation of body temperature or fever. It is a well-known adverse effect of bleomycin when given systemically [29]. This suggests that some of the bleomycin leaks into the bloodstream causing the hypersensitivity reaction that gives rise to the elevated body temperature [29].

Only one other patient was reported to have an adverse effect usually seen when bleomycin is given intravenously and that was a case of hyperpigmentation over bony prominences [9].

Headache

The next most common adverse effect was headache, with at least 30 (16%) patient reports. The headache often presented itself during or directly after the treatment and was transient. None of the studies report whether medication, other than the use of corticosteroids and dose reduction, was necessary in preventing or managing this adverse effect.

Nausea and vomiting

Nausea and vomiting was reported in at least 18 (10%) patients as a direct adverse effect closely related to the treatment. In addition, no treatment besides corticosteroids and dose reduction was suggested in managing this adverse effect.

Peritumoral edema

Peritumoral edema was reported in seven (4%) cases [2,3,9,25], but this number may be underreported for two reasons. It requires a scan of the brain to detect the diagnosis and it is not always symptomatic. The symptoms are often signs of raised intracranial pressure, for example, headaches and vomiting or specific pressure symptoms, such as mydriasis and ptosis, or hemiparesis [25]. Indeed, many of the patients with headache, nausea and vomiting must have had undiagnosed peritumoral edema. It should be considered that the headaches, nausea and vomiting reported could be a direct result of irritation of the brain tissue from bleomycin, or a symptom of peritumoral edema and elevated intracranial pressure. Usually, the edema resolves by treatment with high dose of steroids for a few days, that is, methylprednisolone 30 m/kg/day for 3 days and then progressively tapered over a month [25].

Fatigue

Lethargy, fatigue or somnolence, was reported in at least 10 (5%) cases.

Seizures

Surprisingly, only five (3%) cases of seizures were reported. Two of these were known to have epilepsy before the treatment and their antiepileptic drugs were out of therapeutic range [8]. These are surprisingly few reports, because brain tumors and foreign bodies dispose to epileptic seizures in general.

Miscellaneous adverse effects

In 2000, Savas *et al.* [18] presented the first and only fatal case report directly related to treatment with intracystic bleomycin and indeed this particular patient was treated with extraordinarily high doses. The patient was a 47-year-old woman with a recurrent craniopharyngioma in the third ventricle, who received daily intracavitary injections of bleomycin for 8 days until a total dose of 56 mg (i.e. 100 U) [18]. Five days after conclusion of the treatment the patient deteriorated neurologically, developed secondary hypothyroidism, and lost consciousness in another 2 days. MRI showed diffuse edema in the diencephalon and mesencephalon and the patient died 45 days later. Computed tomographic (CT) scan with contrast showed no leakage before the administration of bleomycin. The authors interpreted the death as a direct toxic effect of bleomycin on the neuroglial tissue. No autopsy was performed.

Broggi *et al.* [7] described a case of complete deafness after 5 mg bleomycin as a single dose. A similar case was also observed by Frank *et al.* [14] who experienced one patient with irreversible neurosensory deafness after an unpublished dose of bleomycin and suggested it was caused by hematological spread. These two cases were published 6 years apart, and Broggi *et al.* [7] did not believe the deafness to be a true adverse side effect of bleomycin, because of no other previous reports in the literature up to that time. Frank *et al.* [14] did not link their case of deafness to the case reported by Broggi *et al.* [7], and call it an ototoxic reaction of bleomycin.

Adverse effects related to the placement of the catheter were observed; for instance, by misplacement and leading to leakage of cystic fluid and bleeding [3,9]. Adverse effects related to the correct placing of the catheter seemed to be limited to four patients: one with skin infection and decubitus, one with skin infection and two with skin ulcers around the injection sites caused by leakage of bleomycin into the skin [8,20]. It should be noted that the last two patients mentioned received total cumulative doses of more than 300 U in an indwelling catheter for more than 17 weeks [20].

Five cases of craniopharyngiomas were reported to have damage to the pituitary gland and hypothalamus caused by bleomycin. Haisa *et al.* [13] presented a case of hypersomnia, personality changes, memory impairment, and thermal dysfunction. Hader *et al.* [3] reported a case of chronic hypopituitarism after peritumoral edema as mentioned earlier. Hukin *et al.* [9] also report a patient with panhypopituitarism at 2 years despite persistent resolution of the cyst. One child developed precocious puberty, although the cyst had shrunk [9]. Ultimately, Lafay-Cousin *et al.* [25] reported a case of a 9-year-old boy who received a cumulative dose of 33 mg (59 U) and developed diabetes insipidus, which was controlled by a minimal dose of desmopressin (12.5 μg/day) for a month and then discontinued.

Bleomycin

Bleomycin is an antibiotic produced from the fungus *Streptomyces verticillus* and was discovered by Umezawa *et al.* in 1966 [30]. It is formed by a mixture of peptides and contains a unique structural component, the bleomycinic acid, and a terminal alkylamine group [27]. As bleomycin is a hydrophilic and charged molecule with a molecular weight of 1500 Da, it passes the intact plasma membrane poorly [31].

Bleomycin is a good chelator of metals and can, in the presence of oxygen, bind to ions of iron, cobalt, zinc, and copper [32,33]. The bleomycin-Fe^{2+} complex is the most active complex [28]. When bleomycin chelates with iron in the presence of oxygen, DNA breaks are induced and lipid peroxidation is mediated by the production of free radicals [27,34].

Bleomycin induces single-strand and double-strand DNA breaks with a ratio of 10 : 1 [27]. It is probably the double-strand breaks and resulting loss of chromosome fragments that are responsible for the cytotoxicity [33]. Therefore, bleomycin has to be internalized in the cell to have an effect; hence, the cell membrane is the limiting factor [31,35]. There are two scenarios depending on how many molecules of bleomycin enter into the cell: (i) the cells are arrested in the G2–M phase of the cell cycle and die in approximately three doubling times with low concentration of bleomycin, and (ii) pseudoapoptosis is induced and kills the cell within minutes with high concentrations of bleomycin [32]. Bleomycin's mechanism of action is not cell cycle specific, though the cells in G2/M phase are more sensitive than the cells in the G1 phase [33].

Bleomycin is used in the treatment of lymphoma (Hodgkin and non-Hodgkin) and testicular cancer in combination with other antineoplastic drugs [2,27,34]. It is also the preferred drug in treating tumors with electrochemotherapy [28,36,37]. Bleomycin is eliminated from the blood by renal excretion [33]. The most important toxic reactions affect the lungs and skin, causing pulmonary fibrosis in about 10%, with a mortality rate of 1%, and erythema, induration, hyperkeratosis, and peeling of the skin [2,27]. The toxicity of bleomycin increases with and is directly related to the cumulative dose received. It should be noted that normally fever occurs 48 h after intravenous drug administration in 25% of the patients [27].

Bleomycin and cobalt

As mentioned above, bleomycin is a good chelator of metal ions such as cobalt. Binding of bleomycin to cobalt is the most stable and therefore irreversible and this binding incapacitates bleomycin's ability to cut DNA [31,33]. The cobalt–bleomycin complex is, therefore, not cytotoxic, and ^{57}Co linked to bleomycin can be used to trace bleomycin and, for example, help to quantify bleomycin inside the cell [38]. When bleomycin binds to ^{60}Co it becomes a radioactive isotope, which can be used as intratumoral radiotherapy and the range of effect is approximately 2–3 cm [1].

Electrochemotherapy

Electroporation or electropermeabilization is a means to facilitate transport of normally nonpermeant molecules into cells [28]. When a pulsed electric field is applied to the cells, the capacitance of the cell membrane is overridden, temporarily destabilized and becomes permeable [35]. Using electroporation to facilitate the uptake of chemotherapy is termed electrochemotherapy.

Electroporation of the cell membrane increases the uptake of bleomycin and therefore cytotoxicity dramatically [39]. Other antineoplastic drugs (e.g. doxorubicin) do not show the same enhancement of cytotoxicity with electroporation, probably because they normally cross the plasma membrane easily on their own, because of their amphiphilic or lipophilic nature [39]. It has been shown that millions of molecules of bleomycin enter the cell by electroporation, which causes cell apoptosis judging by morphological appearance [27]. As bleomycin shortcircuits the apoptosis pathway, the condition has also been termed 'pseudoapoptosis' [32].

Bleomycin can, therefore, become a very effective antineoplastic drug once access to the cytosol is provided. On account of the local placement of electrodes, systemic toxicity may remain limited while an effective treatment zone is created in the area subjected to electric pulses [33]. Electrochemotherapy has been successfully applied to small cutaneous tumors, where bleomycin is given either intravenously or intratumorally, and then electroporated through electric pulses from electrodes in the tumor. The treatment resulted in 75–80% clinical remissions after only one treatment [37].

Discussion

In the treatment of 189 patients with cystic and solid brain tumors with intratumoral bleomycin, five (3%) cases of severe and six (3%) cases of moderate adverse effects (Tables 1 and 3) were observed. Ten out of 11 cases with severe and moderate adverse effects were patients with craniopharyngiomas and probably because of tumor localization in the deep brain.

The cases of severe adverse effects are listed in Table 3, all of which had the diagnosis craniopharyngioma. In the case of craniopharyngioma with a fatal outcome reported by Savas *et al.* [17], we speculate that the generalized brain edema could be caused by the high individual dose and especially the treatment frequency. In the case of bilateral blindness after incorrect dilution of bleomycin reported by Mottolese *et al.* [4], the adverse effect is probably also because of the tumor localization close to the chiasma opticum. The same could be true for the other case of blindness in one eye reported by Belen *et al.* [26].

Craniopharyngiomas are benign epithelial neoplasms originating from the remnants of Rathke's pouch located in the sellar, parasellar, and third ventricular regions and are, therefore, close to structures such as the hypothalamus, the pituitary gland, and the optic nerves in the chiasma opticum [18,15]. Thus, the most adverse effects seen in the studies of treatment of craniopharyngiomas, such as damage to the hypothalamic and pituitary gland leading to, for example, diabetes insipidus and hormone deficiency, can therefore, be explained by the tumor localization.

Table 3 Five cases with severe complications after intracerebral bleomycin related to dose

Studies	Diagnosis	Dose	Severe adverse affects
[18]	Craniopharyngioma	141 U in 8 days	Generalized brain edema leading to death
[4]	Craniopharyngioma	1 incorrectly diluted unknown dose	Blindness
[26]	Craniopharyngioma	141 U in 5 weeks	Blind, one eye
[7]	Craniopharyngioma	Single dose 9 U	Deafness
[14]	Craniopharyngioma	Unknown	Deafness

The two cases of deafness are hard to explain by referral to bleomycin being ototoxic [14], because this is not a known adverse effect of bleomycin. Bleomycin is not normally considered to be ototoxic and it is not mentioned in the literature even as a potential adverse effect [29,34]. It is difficult to determine whether or not this is true because bleomycin is often administered with cisplatin, for instance, in the treatment of testicular cancer (the standard cisplatin/etoposide/bleomycin regimen), which has proven to be a highly ototoxic drug with well-known high incidence of high-frequency hearing loss and tinnitus [29,40].

Six cases of moderate adverse effects were found among the 189 patients treated with bleomycin. Five of these were observed in patients with craniopharyngiomas. In 1994, Haisa *et al.* [13] reported a case of toxic effects on the hypothalamus such as hypersomnia, memory impairment, and thermal dysfunction. Hader *et al.* [3] described a case of hypothalamic–pituitary dysfunction and peritumoral edema verified by CT after treatment with 2 mg bleomycin three times a week for 4 weeks (24 mg i.e. 43 U). The symptoms and the edema resolved over several months, though the patient was left with a permanent worsening of pituitary function [3]. A CT scan with contrast was performed and confirmed no leakage and the authors, therefore, concluded that bleomycin may diffuse through the cyst wall or the effect of the drug on the cyst wall may cause a reaction in the adjacent brain [3].

In a cohort of 17 patients, Hukin *et al.* [9] reported three cases of moderate adverse effects in 2007. Two of the cases had peritumoral edema; one was left with long-term panhypopituitarism, and one continued to suffer from hemiparesis, third brainstem nerve paresis, and decreased attention [9]. The third patient had panhypopituitarism after 2 years, despite resolution of the cyst [9].

All the cases with severe and moderate adverse effects were found in studies of treatment of craniopharyngiomas, except for one. A moderate adverse effect was observed in a patient with glioblastoma who experienced a sudden rise of

intracranial pressure leading to emergency surgery [11]. This is only categorized as a moderate adverse effect as the patient recovered and did well for 5 months until he died of tumor regrowth [11].

Five cases (3%) with questionable adverse effects to treatment with bleomycin were also observed. All five cases were carefully considered and though some were very serious, we find them indeed questionable. In 1989, Broggi *et al.* [7] observed a case of cerebral ischemia in the area of the ipsilateral middle cerebral artery 3 months after treatment with bleomycin, which might be caused by a vasospasm induced by bleomycin. In 1997 Sagoh *et al.* [16] reported a case of occlusive cerebrovasculopathy 19 years after the treatment. The patient had received intracavitary bleomycin combined with internal radiation with ^{198}Au, and did not exhibit any ischemic symptoms. In 2002, Park *et al.* [21] reported a case of cerebellar infarction 2 months after administration of bleomycin and found it to be unclear whether this was because of bleomycin. Park *et al.* [21] also reported a case of a 14-year-old boy who, after only one cycle of bleomycin, remained bed-ridden 6 years after the treatment, which Park *et al.* [21] considered to be a toxic reaction of bleomycin. It should be noted, however, that the patient was already hemiparetic and had a lactate dehydrogenase level over 2000 U, which could be a poor prognostic factor [21], and the case was not described in any further detail. The same year, Jiang *et al.* [23] reported a case of paralysis of the left limbs after an epileptic seizure, which they thought might not have had to do with bleomycin.

An overall rate of severe adverse effects of 3% and of moderate adverse effects of also 3% makes us conclude that the use of bleomycin intratumoral and intracystic for brain tumors is a relatively safe treatment. The most common adverse effects were transient fever, headache, nausea and vomiting, peritumoral edema, fatigue, and seizures. The adverse effects seen in the treatment of craniopharyngiomas was mostly because of the tumors' anatomical proximity to delicate structures in the deep brain. The doses and treatment frequency are, in our opinion, also a big part of the explanation in some cases.

In studies of electrochemotherapy for cutaneous metastases, bleomycin concentrations of either 5 U/ml [41] or 1 U/ml [37] have been used. From a study published by Marty *et al.* [37], it was shown that a dose of 1 U/ml was sufficient and equipotent to intravenous administration or administration of another chemotherapeutic agent (cisplatinum). We would, therefore, recommend that a similar dosing regimen is used in the treatment of brain tumors with intratumoral bleomycin [36]. For a brain tumor of 2.5 cm^3 this would lead to a dose of 0.625 U (1 mg), and for a tumor of 5 cm^3 this would lead to a dose of 1.25 U (2.2 mg). These doses are calculated by using the longest diameter of tumor (a) and (b) the next longest diameter of tumor perpendicular to (a) to calculate tumor volume (V) and the volume formula $V = a \times b \times \pi/6$ and the regimen of 0.25 ml/cm^3 tumor tissue [36]. An alternative is to use intravenous administration of bleomycin, using a standard dose of bleomycin, that is, 15 U/m^2 before electroporation [36]. This has proved effective in one animal study by Salford *et al.* [10] (bleomycin 1 mg/kg intravenously), and has been tried in cutaneous tumors successfully [37]. The question would be whether the BBB would hinder penetration of bleomycin, although it is promising that Front *et al.* have showed the uptake of bleomycin in both primary and secondary human brain tumors [42,43]. It is also highly possible that electroporation of the tumor cells will incapacitate the BBB.

In conclusion, treatment of solid and cystic brain tumors with bleomycin was fairly tolerable and certainly the doses necessary for electrochemotherapy are much smaller than those used in the reviewed studies.

References

1 Nakazawa S, Ohwaki K, Shimura T, Itoh Y, Yajima K. A new treatment of malignant brain tumor-1: local injection of bleomycin (author's transl). *No Shinkei Geka* 1981; **9**:1487–1493.

2 Morantz RA, Kimler BF, Vats TS, Henderson SD. Bleomycin and brain tumors. A review. *J Neurooncol* 1983; **1**:249–255.

3 Hader WJ, Steinbok P, Hukin J, Fryer C. Intratumoral therapy with bleomycin for cystic craniopharyngiomas in children. *Pediatr Neurosurg* 2000; **33**:211–218.

4 Mottolese C, Stan H, Hermier M, Berlier P, Convert J, Frappaz D, *et al.* Intracystic chemotherapy with bleomycin in the treatment of craniopharyngiomas. *Childs Nerv Syst* 2001; **17**:724–730.

5 Kim SD, Park JY, Park J, Lee JB, Kim SH, Lim DJ. Radiological findings following postsurgical intratumoral bleomycin injection for cystic craniopharyngioma. *Clin Neurol Neurosurg* 2007; **109**:236–241.

6 Takahashi H, Yamaguchi F, Teramoto A. Long-term outcome and reconsideration of intracystic chemotherapy with bleomycin for craniopharyngioma in children. *Childs Nerv Syst* 2005; **21**:701–704.

7 Broggi G, Giorgi C, Franzini A, Servello D, Solero CL. Preliminary results of intracavitary treatment of craniopharyngioma with bleomycin. *J Neurosurg Sci* 1989; **33**:145–148.

8 Boiardi A, Eoli M, Pozzi A, Salmaggi A, Broggi G, Silvani A. Locally delivered chemotherapy and repeated surgery can improve survival in glioblastoma patients. *Ital J Neurol Sci* 1999; **20**:43–48.

9 Hukin J, Steinbok P, Lafay-Cousin L, Hendson G, Strother D, Mercier C, *et al.* Intracystic bleomycin therapy for craniopharyngioma in children: the Canadian experience. *Cancer* 2007; **109**:2124–2131.

10 Salford LG, Persson BR, Brun A, Ceberg CP, Kongstad PC, Mir LM. A new brain tumour therapy combining bleomycin with in vivo electropermeabilization. *Biochem Biophys Res Commun* 1993; **194**:938–943.

11 Bosch DA, Hindmarsch T, Larsson S, Backlund EO. Intraneoplastic administration of bleomycin in intracerebral gliomas: a pilot study. *Acta Neurochir Suppl (Wien)* 1980; **30**:441–444.

12 Takahashi H, Nakazawa S, Shimura T. Evaluation of postoperative intratumoral injection of bleomycin for craniopharyngioma in children. *J Neurosurg* 1985; **62**:120–127.

13 Haisa T, Ueki K, Yoshida S. Toxic effects of bleomycin on the hypothalamus following its administration into a cystic craniopharyngioma. *Br J Neurosurg* 1994; **8**:747–750.

14 Frank F, Fabrizi AP, Frank G, Fioravanti A. Stereotactic management of craniopharyngiomas. *Stereotact Funct Neurosurg* 1995; **65**:176–183.

15 Cavalheiro S, Sparapani FVD, Franco JOB, daSilva MC, Braga FM. Use of bleomycin in intratumoral chemotherapy for cystic craniopharyngioma – case report. *J Neurosurg* 1996; **84**:124–126.

16 Sagoh M, Murakami H, Hirose Y, Mayanagi K. Occlusive cerebrovasculopathy after internal radiation and bleomycin therapy for craniopharyngioma – case report. *Neurol Med Chir (Tokyo)* 1997; **37**:920–923.
17 Savas A, Arasil E, Batay F, Selcuki M, Kanpolat Y. Intracavitary chemotherapy of polycystic craniopharyngioma with bleomycin. *Acta Neurochir* 1999; **141**:547–549.
18 Savas A, Erdem A, Tun K, Kanpolat Y. Fatal toxic effect of bleomycin on brain tissue after intracystic chemotherapy for a craniopharyngioma: case report. *Neurosurgery* 2000; **46**:213–216.
19 Disabato JA, Handler MH, Strain JD, Fleitz JM, Foreman NK. Successful use of intracavitary bleomycin for low-grade astrocytoma tumor cyst. *Pediatr Neurosurg* 1999; **31**:246–250.
20 Patchell RA, Regine WF, Ashton P, Tibbs PA, Wilson D, Shappley D, *et al*. A phase I trial of continuously infused intratumoral bleomycin for the treatment of recurrent glioblastoma multiforme. *J Neurooncol* 2002; **60**:37–42.
21 Park DH, Park JY, Kim JH, Chung YG, Lee HK, Lee KC, *et al*. Outcome of postoperative intratumoral bleomycin injection for cystic craniopharyngioma. *J Korean Med Sci* 2002; **17**:254–259.
22 Alen JF, Boto GR, Lagares A, de la LA, Gomez PA, Lobato RD. Intratumoural bleomycin as a treatment for recurrent cystic craniopharyngioma. Case report and review of the literature. *Neurocirugia (Astur)* 2002; **13**:479–485.
23 Jiang R, Liu Z, Zhu C. Preliminary exploration of the clinical effect of bleomycin on craniopharyngiomas. *Stereotact Funct Neurosurg* 2002; **78**:84–94.
24 Nicolato A, Foroni R, Rosta L, Gerosa M, Bricolo A. Multimodality stereotactic approach to the treatment of cystic craniopharyngiomas. *Minim Invasive Neurosurg* 2004; **47**:32–40.
25 Lafay-Cousin L, Bartels U, Raybaud C, Kulkarni AV, Guger S, Huang A, *et al*. Neuroradiological findings of bleomycin leakage in cystic craniopharyngioma-Report of three cases. *J Neurosurg* 2007; **107**:318–323.
26 Belen D, Er U, Yigitkanli K, Bolay H. Delayed neurotoxic complication of intracavitary bleomycin therapy for craniopharyngioma in a child who had previously undergone radiosurgery – Case report. *J Neurosurg* 2007; **106**:391–393.
27 Lazo J, Chabner B. Bleomycin. In: Chabner B, Longo D, editors. *Cancer chemotherapy and biotherapy*. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1996. pp. 379–393.
28 Gothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat Rev* 2003; **29**:371–387.
29 Chu E, De Vita V. *Physicians' cancer chemotherapy drug manual 2007*. Sudbury, Masachusetts: Jones and Bartlett Publishers; 2007.
30 Umezawa H, Maeda K, Takeuchi T, Okami Y. New antibiotics, bleomycin A and B. *J Antibiot (Tokyo)* 1966; **19**:200–209.
31 Poddevin B, Orlowski S, Belehradek J, Mir LM. Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culture. *Biochem Pharmacol* 1991; **42**:S67–S75.
32 Tounekti O, Pron G, Belehradek J Jr, Mir LM. Bleomycin, an apoptosis-mimetic drug that induces two types of cell death depending on the number of molecules internalized. *Cancer Res* 1993; **53**:5462–5469.
33 Mir LM, Tounekti O, Orlowski S. Bleomycin: revival of an old drug. *Gen Pharmacol* 1996; **27**:745–748.
34 Bokemeyer C. Bleomycin in testicular cancer: will pharmacogenomics improve treatment regimens? *J Clin Oncol* 2008; **26**:1783–1785.
35 Heller R, Gilbert R, Jaroszeski MJ. Clinical applications of electrochemotherapy. *Adv Drug Deliv Rev* 1999; **35**:119–129.
36 Mir LM, Gehl J, Sersa G, Collins CG, Garbay JR, Billard V, *et al*. Standard operating procedures of the electrochemotherapy: instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the Cliniporator (TM) by means of invasive or non-invasive electrodes. *Eur J Cancer Suppl* 2006; **4**:14–25.
37 Marty M, Sersa G, Garbay JR, Gehl J, Collins CG, Snoj M, *et al*. Electrochemotherapy – an easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. *Eur J Cancer Suppl* 2006; **4**:3–13.
38 Poddevin B, Belehradek J, Mir LM. Stable [Co-57] Bleomycin complex with a very high specific radioactivity for use at very low concentrations. *Biochem Biophys Res Commun* 1990; **173**:259–264.
39 Gehl J, Skovsgaard T, Mir LM. Enhancement of cytotoxicity by electropermeabilization: an improved method for screening drugs. *Anticancer Drugs* 1998; **9**:319–325.
40 Fossa SD, de WR, Roberts JT, Wilkinson PM, de Mulder PH, Mead GM, *et al*. Quality of life in good prognosis patients with metastatic germ cell cancer: a prospective study of the European organization for research and treatment of cancer genitourinary group/medical research council testicular cancer study group (30941/TE20). *J Clin Oncol* 2003; **21**:1107–1118.
41 Heller R, Jaroszeski MJ, Reintgen DS, Puleo CA, DeConti RC, Gilbert RA, *et al*. Treatment of cutaneous and subcutaneous tumors with electrochemotherapy using intralesional bleomycin. *Cancer* 1998; **83**:148–157.
42 Front D, Even-Sapir E, Iosilevsky G, Israel O, Frenkel A, Kolodny GM, *et al*. Monitoring of 57Co-bleomycin delivery to brain metastases and their tumors of origin. *J Neurosurg* 1987; **67**:506–510.
43 Front D, Israel O, Iosilevsky G, Evensapir E, Frenkel A, Kolodny GM, *et al*. SPECT quantitation of cobalt-57 bleomycin delivery to human-brain tumors. *J Nucl Med* 1988; **29**:187–194.