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ORIGINAL ARTICLE

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Ocular symptoms in children treated with human-mouse chimeric anti-GD2 mAb ch14.18 for neuroblastoma

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Abstract Unusual ocular symptoms observed during intravenous treatment with anti-disialoganglioside antibody (Ab) in children suffering from neuroblastoma were analyzed and the results reported. Within the framework of the German Collaborative Neuroblastoma Study NB97, 85 children with high-risk neuroblastoma received anti-GD2 monoclonal antibody ch14.18 intravenously. Side effects were regularly reported to the study center. Ocular symptoms were recorded in clinical detail, duration and development over time. Symptoms of a parasympathetic deficit corresponding to internal ophthalmoplegia, i.e. mydriasis and accommodation deficit, were found in 10 patients. They were uni- or bilateral, began after the termination of Ab infusion and improved or disappeared in all surviving children. They did not reappear or worsen upon repeated Ab infusions. The pathophysiology of these disorders remains poorly understood. It is concluded that during systemic treatment with the anti-GD2 antibody ch14.18, reversible symptoms of parasympathetic denervation of the eye may occur which, however, do not warrant termination of this treatment.

Keywords Anti-GD2 monoclonal antibody · Neuroblastoma

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Introduction

Neuroblastoma is the most frequently occurring solid tumor in childhood and ranks third after leukemias/lymphomas and central nervous system (CNS) tumors in the incidence of childhood malignancies. The cumulative incidence in Germany is 16.9 cases per 100,000 children aged less than 15 years.

Thirty-eight percent of patients are considered high-risk based on the criteria of disseminated disease (stage 4) or amplification of the *MYCN* oncogene in the tumor cells, irrespective of stage. Only 35% of these patients remain disease-free after treatment, despite high-dose chemotherapy and autologous bone-marrow transplantation [1]. Therefore, new treatment approaches are needed to control residual disease after chemotherapy.

Ganglioside GD2 is strongly expressed on human neuroblastoma cells. In addition, it is found on the cell surface of melanomas, gliomas, small cell lung cancers and some osteosarcomas [11]. Studies with a murine monoclonal anti-GD2 antibody (14.G2a) and a chimeric human/murine anti-GD2 antibody (ch14.18) have shown that both induce antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity of neuroblastoma cells in humans [5, 6]. Human anti-mouse Abs were rarely observed in patients treated with the chimeric antibody ch14.18, which was then further evaluated in phase I and pharmacokinetic studies [3, 7, 9]. In 1997, the German Cooperative Neuroblastoma Group included ch14.18 within a cooperative treatment protocol, NB97. Six courses of antibody treatment are given over 12 months after completion of drug chemotherapy in high-risk patients. This study is still ongoing [1].

Well-known side effects of intravenous application of ch14.18 are visceral pain, allergic reactions, nausea, vomiting, fever, hypotension and neurotoxicity. However, ocular side effects are reported as rare events. We therefore evaluated ocular symptoms in children treated within the study NB97 with ch14.18 from 1997 until 1999.

Patients and methods

From May 1997 until December 1998, 107 children with high-risk neuroblastoma (stage 4 disease and *MYCN* oncogene amplification at any stage) were treated within the German Cooperative Neuroblastoma Study NB97; 85 of the 107 patients received at least one treatment schedule of ch14.18 after the completion of cytostatic treatment (a detailed description of this protocol is given [1]). The antibody was obtained from BioInvent International AB (Lund, Sweden) and GMP-certified by the Paul-Ehrlich-Institute (Langen, Germany). It was administered at a dose of 20 mg/m^2 per day as an 8-h intravenous infusion for 5 consecutive days. Concomitantly, intravenous morphine hydrochloride was given, usually 1.0 mg/kg/24 h with dose adjustment to control visceral pain. This 5-day treatment schedule was repeated every 2 months for a total of six courses at most. Side effects of the Ab treatment were regularly reported to the study coordinators.

Ocular symptoms were reported in 11 children. Detailed investigation revealed iatrogenic mydriasis before fundoscopy in one child, who was then excluded from this analysis. This report refers to the other ten patients, i.e. two girls and eight boys with a median age of 49 months at diagnosis of neuroblastoma (range: 16–72 months). Nine subjects had disseminated disease (stage 4), while one boy was treated for a relapse of stage 3 neuroblastoma. Investigation of these patients included the interval from diagnosis until the onset of ocular symptoms, specific description and laterality of symptoms, simultaneous side effects other than ocular, duration and development of symptoms and preexisting ocular symptoms or tumor involvement of the CNS or orbita.

Results

The time from diagnosis of neuroblastoma to the onset of ocular symptoms was 16 months on average (range: 8–20 months; cf. Table 1). This was due to the experimental protocol with the Ab treatment scheduled at the end. In detail, the symptoms were the following (cf Table 2): mydriasis was observed in nine out of ten patients and was unilateral in five and bilateral in four cases. In patient 8, who showed left-sided pupillotonia, both pupils were found to be hypersensitive to pilocarpine 0.1%, which is indicative of parasympathetic denervation. One child experienced an isolated bilateral accommodation deficit (4 dpt instead of 12; patient 5). Four other children showed an accommodation deficit in addition to mydriasis (patients 2, 3, 4 and 7). The ocular symptoms occurred in relation to the first course of Ab in four out of 10 patients (nos. 1, 3, 4 and 9), after the fourth course in three patients (nos. 5, 7 and 8) and after the third, fifth and sixth course in children 10, 6 and 2, respectively. These symptoms were detected on day 5 during the first treatment course in patient 1; in all the others, they began after completion of the course from day 1 up to 2 months post-therapy. The symptoms were observed for periods ranging between 2 and 12 months. Five children recovered completely, four of them without any further treatment, one after steroid administration. Four of the patients died from neuroblastoma with no further

Table 1 Patient characteristics. The ocular symptoms and cerebral findings at diagnosis of neuroblastoma, mainly due to metastases, had resolved by the time the patients were treated with mAb ch14.18

	Sex	Age at diagnosis (mo)	Stage	Ocular symptoms during treatment with mAb ch14.18 (age in mo)	Ocular symptoms at diagnosis	Cerebral or ocular involvement
1 DJ	M	65	Relapse after 3	85	None	None
2 DM	F	72	4	92	None	None
3 HY	M	55	4	63	None	Cerebral involvement
4 IG	M	67	4	77	Bilateral orbital hematoma	Bilateral orbital involvement
5 UB	M	65	4	77	Proptosis of right eye	Right orbital involvment, diffuse infiltrates of whole skull
6 MD	M	42	4	60	None	None
7 RM	M	18	4	35	None	Bilateral orbital involvement
8 SR	F	32	4	51	None	None
9 SJ	M	16	4	29	None	None
10 SK	M	19	4	33	None	None

Table 2 Ocular symptoms at time of treatment with anti-GD2 mAb ch14.18 (0: normal, +: improved, = no change, – worsened)

Case no.	Symptoms	Laterality	mAb ch.14.18 treatment-related onset of symptoms	Duration (months)	Status[a]
1	Mydriasis	B	Course 1, day 5	3	0
2	Mydriasis, accommodation deficit	R	Course 6, day 1 after end	2	0; Rapid improvement
3	Mydriasis, accommodation deficit	B	Course 1, day 1 after end	6+	Partial improvement
4	Mydriasis, accommodation deficit	L	Course 1, 3 weeks after end	5+	=; Death from NB
5	Accommodation deficit 4 dpt (nl: 12 dpt)	B	Course 4, day 4 after end	9	0
6	Mydriasis	L	Course 5. 2 mo after end	11	=; Death from NB
7	Mydriasis, accommodation deficit	R	Course 4, 3 weeks after end	12	=; Death from NB
8	Mydriasis (asymmetrical)	B	Course 4, day 3 after end	5	0
9	Mydriasis	B	Course 1, 3 weeks after end	11	0; Rapid improvement
10	Mydriasis	R	Course 3, 2 weeks after end	3	=; Death from NB

change in the ocular symptoms. One boy (patient 3) has survived with an improvement of mydriasis and accommodation deficit which has been apparent for 6+ months.

Treatment with ch14.18 was continued in three of the children (patients 3, 7 and 8). Patient 7 received an additional course without any worsening of ocular symptoms but finally died of disease progression. Patient 8 received two additional courses; the ocular symptoms regressed slowly and finally disappeared despite continued administration of the Ab. Patient 3 was given three further courses of the Ab; he experienced slow improvement with some residual accommodation deficit and mydriasis during the treatment period.

All children with ocular symptoms during treatment with ch.14–18 were investigated for the presence of previous ocular or cerebral symptoms or extraocular neurological findings.

Bilateral periorbital ecchymosis was reported in patient 4, and proptosis of the right eye in patient 5. Orbital involvement with neuroblastoma was found at diagnosis in only three out of 10 patients (Table 1). Abnormalities detected by imaging studies of the central nervous system (CNS) included cerebral metastases in two children, meningeal enlargement on MRT scan in one case and a decreased density of the right nucleus lentiformis in another patient. Other neurological disorders were the following: patient 5 had right facial palsy; patient 9 had fine motor deficits; and a third patient (7) showed intermittent non-specific EEG changes. All these findings, which dated from the time of diagnosis, were mostly due to metastases and had resolved by the time the patients were treated with Ab ch14.18.

Discussion

Ocular symptoms have rarely been mentioned in the literature when the side effects of treatment with anti-GD2 Abs are described. Of a total of nine children with neuroblastoma who received 19 courses of anti-GD2 Ab, transient pupillotonia was described in one child (while two others had unilateral atrophy of the optic nerve after local radiotherapy [3]). In a study of 11 patients with neuroblastoma and osteosarcoma, Yu et al. mentioned one case of ptosis of the left eye which was, however, attributed to morphine dosage [11]. During another trial, 19 children received 79 courses of ch14.18 treatment for neuroblastoma. Dilated pupils were described in 6 of them, 4 of whom also experienced blurred vision. All symptoms resolved over a period of days to weeks [7]. No eye symptoms at all were reported in 34 children with neuroblastoma treated with an anti-GD2 Ab, 3F8, by Cheung et al. [2].

Our report investigated the largest group of patients with ocular symptoms after treatment with anti-GD2 Ab described so far. In all patients in the present study, ocular symptoms corresponded to a parasympathetic ocular deficit. We found that mydriasis and accommodation deficit were the only ocular symptoms. They were unilateral or bilateral and in one case asymmetrically bilateral, as demonstrated by hypersensitivity to pilocarpine 0.1%. This observation, together with the fact that pupillotonia could be detected either in one or in both eyes may indicate that the unilateral disorders were in fact bilateral, but that the deficit was too small to be detected by mere examination. No other explanation for a unilateral expression of a systemic side effect could be suggested.

In our group of patients, the above-described symptoms lasted from several months to up to 1 year, and in the six surviving patients they either resolved completely or improved (in one child) during the observation period. The symptoms began during anyone of the consecutive treatment courses; in no case, however, immediately after the onset of the infusion, but either at day 5, or mostly after termination of the course. In the children who received subsequent treatment courses, the

symptoms either did not worsen or did not reoccur. This finding is in complete contrast to the occurrence of neuropathic pain, which is regularly caused by each infusion of anti-GD2 Ab.

The question arises of whether such ocular symptoms could be caused by an Ab directed against GD2. Disialogangliosides are widely distributed in the CNS. Anti-GD2 Abs administered by the intravenous route do not seem to cross the blood–brain barrier. Studies with radiolabeled antibodies in mice do not show a significant uptake in normal brain [4]. Even more convincingly, an ineffective blood–brain barrier against such Abs would result in significant neurological side effects, which in this study were not observed. Regarding the eye, the retina seems to be included in protection by the blood–brain barrier. No structural or functional damage to the retina attributable to anti-GD2 treatment has ever been described.

However, the presence of GD2 has also been demonstrated in the peripheral motor and sensory nerve fibers [8]. Using immunohistochemistry, GD2 has been detected in the ciliary muscle and in the iris, both of which are of mesodermal origin (R. Bachmann, personal communication; cited in [3]). It can be presumed that the structures that express GD2 are neural structures within the ciliary or sphincter muscle of the iris. The side effects of anti-GD2 treatment in the peripheral nervous system have been well documented. The intravenous infusion of anti-GD2 antibody in patients regularly leads to immediate pain and mechanical allodynia. The pathophysiological expression of these symptoms is reflected by an increased background activity in Aδ and C nerve fibers, as has been shown in a rat model [10]. An increased activity of the sympathetic fibers could explain the mydriasis, but not the accommodation deficit. Both symptoms together only point to decreased nerve activity, namely, that of the parasympathetic fibers. Thus, a common pathophysiological denominator is lacking for the neuropathic pain and the parasympathetic ocular deficit.

The ocular symptoms were observed at the end or after the termination of a 5-day treatment course with anti-GD2 antibody. One hypothesis could be that the morphine administered concomitantly with the Ab counteracted the mydriatic effect in all patients without ocular symptoms. However, this does not explain why the symptoms neither occurred reproducibly during each treatment course nor regularly in both eyes. Also, the symptoms became apparent several weeks after the infusion of morphine and the anti-GD2 Ab, at a time when the effect of morphine upon the ciliary muscle could no longer be presumed.

Neuroblastoma involvement of the orbital or neural structures can lead to ocular symptoms such as Horner's triad or opsomyoclonus. However, such an involvement was demonstrated in only four out of our ten patients with ocular symptoms after anti-GD2 treatment and thus cannot be solely responsible for all the ophthalmic features observed.

In summary, mydriasis or accommodation deficit irregularly occurs after intravenous treatment with anti-GD2 antibody. Taken together, the symptoms correspond to parasympathetic denervation (pupillotonia = Adie's syndrome). Their time course is variable, but symptoms improve in all the patients who survive, even with repeated administrations of the Ab. The symptoms may be unilateral or bilateral, and are not associated with neuroblastoma involvement of the orbital structures. Their pathophysiology can only be speculated upon. Termination of anti-GD2 treatment as a result of such symptoms does not seem to be warranted.

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