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Original

Simultaneous radiotherapy and radioimmunotherapy of malignant gliomas with anti-EGFR antibody labelled with iodine 125. Preliminary results

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Abstract

BACKGROUND: In this paper we present the preliminary results of a prospective trial of the efficacy of simultaneous radiotherapy and anti-EGFR ^{125}I radioimmunotherapy of malignant gliomas with 2 years' total survival as the end-point, raising the question whether anti-EGFR ^{125}I radioimmunotherapy influences the disease-free survival in these patients.

MATERIAL AND METHODS: Patients with anaplastic astrocytoma or primary glioblastoma were previously treated by a macroscopically radical neurosurgical approach and randomized either to radiotherapy + radioimmunotherapy arm or treated by radiotherapy alone. Seven patients were included in the group with radioimmunotherapy, among them five with GBM and two with AA, and five patients in the control arm. Patients were irradiated to 60 Gy using three-dimensional conformal non-coplanar techniques. Anti-EGFR ^{125}I monoclonal antibody 425 radioimmunotherapy (50 mCi/course) was started during 4th week of radiotherapy and was repeated three times in one week intervals.

RESULTS: Time of follow-up ranges between 2 and 10 months in the anti-EGFR ^{125}I radioimmunotherapy arm and 4 and 9 months in the control arm. Recurrence was diagnosed in all patients in the EGFR ^{125}I group with a lethal outcome in two of them and in 4 patients in the control group. Median time to recurrence was 2 and 5 months respectively.

CONCLUSIONS: Taking into account early recurrences observed, we propose to continue the studies on the efficacy of adjuvant anti-EGFR ^{125}I radioimmunotherapy in a selected group of patients in whom the greatest benefit may be expected on the basis of molecular studies, among them EGFR expression investigation.

Key words: radioimmunotherapy, monoclonal antibody 425, epidermal growth factor receptor, iodine 125, glioblastoma, anaplastic astrocytoma

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Introduction

New treatment modalities are necessary in high grade gliomas — anaplastic astrocytomas and glioblastomas — as these brain tumours have a very poor prognosis despite the radical neurosurgical treatment and adjuvant radiotherapy. Systemic chemotherapy also has no major impact. Median survival in primary glioblastomas does not exceed 40 weeks and depends strongly on the extent of operation [1, 2]. However, even when radical surgery and radiotherapy are applied, tumours tend to recur within centimetres of their original location. In patients treated in our institute, the one-year total survival is 50% in anaplastic astrocytoma and only 20% in glioblastoma [3].

Radioimmunotherapy with monoclonal antibodies gives an alternative for complementary therapy of residual disease and may be directed against various antigens present in tumour tissue. Specific delivering of radioisotope into tumour cells may help to destroy residual glioma cells left after macroscopically radical surgery. In recent years, monoclonal antibodies against various antigens, among them epidermal growth factor receptor (EGFR), tenascin or neural cell adhesion molecule, labelled with a variety of isotopes (131-I, 90-Y) have been analysed for their ability to prolong survival and prevent the recurrence in malignant gliomas [4–6].

Brady et al. have proposed the use of monoclonal antibodies against epidermal growth factor receptor, labelled with iodine-125 [7, 8]. The monoclonal antibody 425, developed at the Wistar Institute, is an IgG 2a antibody which becomes internalised when bound to the membrane-receptor complex [9]. It causes downregulation of EGFR without stimulation of tyrosine kinase activity [10]. Overexpression of EGFR is found in many tumours, among them in primary glioblastomas. In some of them even a 100-fold increase of the expression may be noted; what is more, EGFR expression is infrequent in normal brain cells [11]. Thus, EGFR molecules may be a target of anti-glioma therapy [12–15].

The EGFR-antibody 425 complex is internalised and its specific nuclear binding has been observed [10, 16]. The internalisation enables the use of ^{125}I as a radiation source. It is well known that radioactive ^{125}I is a highly effective therapeutic agent, emitting a cascade of Auger electrons with a high linear energy transfer (LET) but due to their low range it may be useful only when a mechanism delivering the isotope in the close vicinity of nuclear DNA is present [17, 18]. High grade gliomas are radio-resistant, which is thought to be a final result of many processes, among them tumour hypoxia, necrosis, and accumulation of sublethal damage. The use of a high LET isotope decreases sublethal and potentially lethal DNA damage repair and is less affected by hypoxia and cell cycle changes [16]. This may ensure a more effective cytoxicity of radioimmunotherapy.

Brady et al. [7] reported in their phase II trial 60% survival at one year for both anaplastic astrocytoma (AA) and primary glioblastoma (GBM) after anti-EGFR ^{125}I radioimmunotherapy. In a subsequent report [8] they compared combined therapy, including conventional neurosurgery, radiation therapy and anti-EGFR ^{125}I radioimmunotherapy versus surgery and radiotherapy. Radioimmunotherapy was given 4–6 weeks after radiotherapy and resulted in a similar total survival in AA and GBM.

Basing on these results, we have started a prospective trial of the efficacy of simultaneous radiotherapy and anti-EGFR ^{125}I radioimmunotherapy of malignant gliomas with 2 years' total survival as the end-point. In the paper we present the preliminary results of the study, raising the question whether anti-EGFR ^{125}I radioimmunotherapy influences the disease-free survival in these patients. As no distinct early influence could be observed, we propose to select patients for further radioimmunotherapy and analyse the potential selection criteria.

Material and methods

Monoclonal antibody and its radioiodination

The 425 monoclonal antibody is a murine Ig 2a derived from the A431 human epidermoid carcinoma cell line [9] and was obtained from one of us (L. Brady) as a 10 mg/ml solution in saline. 5 mg of antibody were used for each radioiodination with Iodogen method, performed in a closed system glove box working under lowered pressure [7]. First, tubes were coated with Iodogen by evaporation of 0.625 ml of a chloroform solution containing 0.5 mg/l of Iodogen, then the 425 monoclonal antibody was added and this was immediately followed by the addition of 60–70 mCi of natrium iodide ^{125}I (OPIDI, Poland) to the reaction tube. The iodination was allowed to proceed for 10 min at room temperature and was terminated with the addition of 0.5 ml of an ascorbic acid solution (10 mg/ml). The radioiodinated protein was then passed through a Sephadex G-25 gel column (PD10) which was prewashed with 15 ml of a sterile 1% HSA (human serum albumin in saline) solution. The final product was eluted off the column with 3.5 ml of HSA solution. After filtering the product through a 0.22 μ filter into a sterile vial, serial dilutions were prepared and measured in a multichannel analyser, allowing for estimation of total radioactivity of the obtained labelled antibody. Radiochemical purity was controlled by thin layer chromatography. The ^{125}I labelled monoclonal antibody was used within 24 h in the first patients, in the next courses of therapy it was prepared immediately before administration.

Patients

The study protocol was approved by the local ethics committee and all patients were accepted after appropriate consent. Among 12 patients enrolled until now there were 8 men and 4 women aged 32 to 58 years. All had AA or GBM confirmed by postoperative histopathological examination (4 cases and 8 cases respectively). Patients were previously treated by a macroscopically radical neurosurgical approach. Only patients showing none or less than 2 ml of remnant tumour on post-operative MR study were enrolled and randomized either to radiotherapy + radioimmunotherapy arm or treated by radiotherapy alone. No patient was postoperatively treated by chemotherapy.

Until now, 7 patients were included in the group with radioimmunotherapy, among them five with GBM and two with AA, and 5 patients in the control arm (Table 1).

Table 1. Patient data

	Anti-EGFR ^{125}I + teleradiotherapy	Teleradiotherapy
No of patients	7	5
Age	32–57 years	46–58 years
Primary glioblastoma	5	3
Anaplastic astrocytoma	2	2

Combined radiotherapy and radioimmunotherapy

Patients were irradiated to 60 Gy using three-dimensional conformal noncoplanar techniques. Low doses of dexamethasone and mannitol were used to prevent brain oedema.

Anti-EGFR ^{125}I radioimmunotherapy was started during the 4th week of radiotherapy, not later than 8 weeks after neurosurgery and was repeated three times in one week intervals. The radioactive antibody solution with total activity approaching 50 mCi in a volume of 20 ml of saline was administered by an infusion pump with appropriate care taken during administration to prevent spillage. The infusion rate was 0.3 ml/min. At that time, all patients were hospitalised at the radionuclide therapy station with facilities enabling collection of ^{125}I — contaminated urine and other radioactive waste. Dose rate measurements were performed 4 times during the first three days after each administration, then twice daily by a dose rate radiometer over 9 skull points with special attention to the tumour bed and appropriate contralateral control side, as well as over thyroid, heart and right wrist.

Standard Lugol potassium iodide solution was administered prior to and during the first two days after each course of radioimmunotherapy in order to prevent dissociated radioactive iodine thyroid uptake.

Follow-up

Control examinations were performed in our department every month. MRI examination was performed every three months or on demand. It was widened by acquisition of MRI-localised ^{1}H-MR spectra for choline, creatinine, N-acetyl aspartate, myoinositol, lactate and lipids [19, 20]. When tumour recurrence was diagnosed, the subsequent treatment was chosen on an individual basis, including secondary neurosurgery or stereotactic radiotherapy (6–9 Gy). Complete blood counts and biochemical profile were checked up. TSH and fT4 were also measured during follow-up for the evaluation of possible thyroid injury.

Results

There were no immediate side effects of anti-EGRF ^{125}I radioimmunotherapy performed or any change in the tolerance of simultaneous teleradiotherapy. The monitoring of anti-EGRF ^{125}I radioimmunotherapy, performed by serial measurements of dose rate over the chosen points of the body with the comparison of the measurements over tumour bed and over the contralateral skull side, was equivocal, giving no indices of specific uptake (Fig. 1). The difference between the involved and the control side of skull was negligible and this could be attributed to the low penetration of the emitted radiation. In one patient an approach to visualise the tumour bed uptake by post therapy scintigraphy was performed, using Nucline one-head gamma-camera equipped with software for ^{125}I. No focus of specific uptake could be acquired.

Time of follow-up ranges between 2 and 10 months in the anti-EGRF ^{125}I radioimmunotherapy arm and 4 and 9 months in the control arm (Table 2). Among seven patients treated postoperatively with teleradiotherapy and anti-EGRF ^{125}I radioimmunotherapy, recurrence was diagnosed in all, with a lethal outcome in two of them. The quick progression observed precluded any specific diagnostic procedures in these two patients and only symptomatic therapy was applied. Five other recurrences were diagnosed by MR, combined with ^{1}H spectroscopy, at 1–2 months after the end of treatment and two of them were accompanied by neurological symptoms. Median time to recurrence was 2 months. Three patients received stereotactic radiotherapy as the secondary line of treatment. One patient (patient No 4) was reoperated after 1 month and suffered from a second recurrence after a further 6 months, diagnosed with MRI, which was treated by stereotactic conformal radiotherapy. The status of all five living patients is now stable.

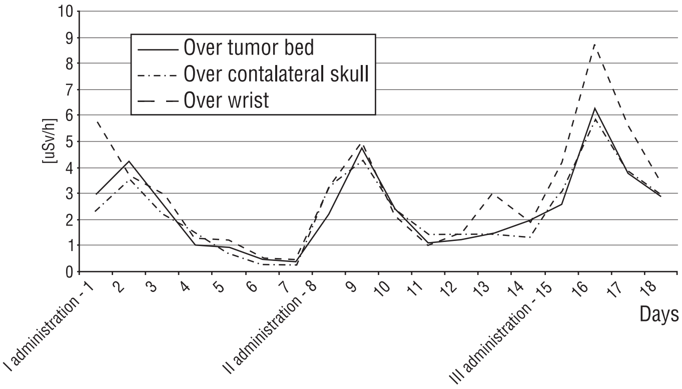

Figure 1. Dose rate measurements during anti-EGFR ^{125}I radioimmunotherapy.

In the control group the follow-up period ranges between 4 and 9 months. Local recurrence was diagnosed by MRI in all but one patient, with whom the contact was lost (Table 2). The median time to recurrence was 5 months with the range between 4 and 7 months. One of them died after 4 months.

The evaluation of TSH and fT4 levels detected subclinical hypothyroidism in one patient in the treated group (TSH: 7 mU/l). Substitutive thyroxine therapy was introduced.

Table 2. Early results of anti-EGFR ^{125}I radioimmunotherapy

Patient No	Local recurrence	Time to recurrence (months)	Decease	Total period of observation (months)
Anti-EGFR ^{125}I radioimmunotherapy + teleradiotherapy				
1	Yes	2	Yes	2
2	Yes	1	Yes	1
3	Yes	6	No	10
4	Yes	1	No	8 (second recurrence observed 6 months after secondary neurosurgery)
5	Yes	2	No	7
6	Yes	2	No	5
7	Yes	2	No	3
Teleradiotherapy alone				
1	No data	No data	No data	No data
2	Yes	5	No	6
3	Yes	4	Yes	4
4	Yes	7	No	9
5	Yes	6	No	9

Discussion

The former studies of anti-EGRF antibody 425 ^{125}I radioimmunotherapy performed after neurosurgery and radiotherapy [7, 8] indicated an increase in total survival of malignant glioma patients to the median of 57 weeks. 50 mCi of anti-EGRF ^{125}I monoclonal antibodies appeared as an optimal and well tolerated activity for a single course of radioimmunotherapy and three courses of therapy ensured the sufficient total activity given. The rationale was to destroy the microfoci of malignant glioma which had been left after surgery in the surrounding normal brain tissue and had not been reached by radiotherapy.

Analysing points which might be important to improve the outcome, we decided to perform anti-EGRF ^{125}I radioimmunotherapy during, not after, teleradiotherapy. The analysis of the putative radiation dose absorbed during radioimmunotherapy course indicates that it does not exceed 5–7 Gy and may even be lower. There was no risk of increasing the potential harm to healthy brain tissue as EGFR is not expressed on normal neurones [11].

Unfortunately, the possibilities to monitor the ^{125}I therapy are much poorer than for ^{131}I, where it is possible to confirm the selective uptake of the labelled antibody in the tumour foci by the post-therapy scintigraphy. We applied activities of ^{125}I in the range up to 50 mCi/treatment. This resulted in the distinct increase in the dose rate measurements, differing over the particular parts of the body and very distinct over the skull (Fig. 1). However, the difference in focal uptake over the tumour bed and the control site could not be confirmed. This may be due to several reasons. First, the fraction of the administered labelled antibody, concentrated by the tumour cells, was rather small. It has been evaluated in other studies as less than 0.1–1% and we estimated it with a small activity of anti-EGRF ^{131}I in a similar range (data not shown). We were also not able to visualise the ^{125}I uptake during post-therapy scintigraphy despite equipping our gamma-camera with software enabling measurements under 30 keV. Considering the high absorption rate of low energy gamma radiation of ^{125}I (during decay ^{125}I emits low energy photons, 3.8–31 keV), we do not interpret the lack of difference in dose rate measurements over the tumour bed and the contralateral side of the skull as evidence of lack of accumulation of the tracer. We rather think that post-therapeutic measurement of anti-EGRF ^{125}I Mab is not possible even with high activities of the isotope applied.

Our study has been planned for 30 patients in each arm with the evaluation of the total survival as the endpoint. Quick recurrences, observed in the patients treated until now, prompt us to reanalyse the design of the study. In the group of patients treated with anti-EGRF ^{125}I radioimmunotherapy the median time to recurrence was 2 months, although they were carefully chosen on the basis of postoperative MR and ^{1}H spectroscopy study as having none or only small (less than 2 ml) residual tumour mass. The former reports of one of us [3,19] clearly indicate that ^{1}H spectroscopy predicts the outcome of postoperative radiotherapy for malignant gliomas with high specificity. Although both experimental and control group are rather small, this may indicate the failure of anti-EGRF ^{125}I radioimmunotherapy.

Lack of any prolongation of the disease-free survival stimulates us to optimise the criteria of selection of patients to whom anti-EGFR therapy should be offered. The EGFR expression in malignant gliomas was described in 37–90% in earlier reports [11]. Considering the high expression level, no immunohistochemical confirmation of its presence was performed in the previous radioimmunotherapy studies [8] and required by our initial protocol. However, recent reports by RT-PCR and microarray DNA expression profiling indicate that EGFR amplification gene is limited only to primary glioblastomas and may be stated at the lower limit of the above range — only in 40% of cases [21, 22]. Although some tumours, which are negative for amplification of the gene, do show expression of EGFR, an implication that every grade III or IV glioma may be reached by anti-EGFR therapy may be improper. Q-PCR or immunohistochemistry based evidence of its overexpression should be required for the primary selection of eligible patients.

Malignant gliomas are very heterogeneous from the molecular point of view [22, 23]. Recent reports on the expression profiling [21, 24] with DNA microarrays have confirmed that primary and secondary glioblastomas are different diseases, they showed also a distinct difference in expression profile between anaplastic astrocytoma and primary glioblastoma. In primary glioblastoma, typical genetic abnormalities included p16/p14 deletion in 40% of cases, EGFR amplification in 40%, LOH on chromosome 10, PTEN mutations in 30% of cases and increased telomerase activity in 60% of cases [21]. Overexpression of EGFR in primary glioblastomas was a good predictor of poor outcome in patients negative for p53 inactivation [23]. Another study, using laser capture microdissection, indicated that EGFR receptor was not upregulated in the invading rim, when compared to the tumour core [24]. This observation may be of significance for the lack of early effect, observed in our study, as invading rim cells were the target of our radioimmunotherapy trial. EGFR gene deletions, described in human glioblastomas, should also be considered [25].

The previous studies exhibited equal results in primary GBM and AA [8], however, taking into account the above mentioned molecular considerations, the indications for anti-EGFR based therapy are more distinct in primary GBM than AA. Among other failure reasons one could consider also EGFR gene polymorphism at Tyr992, as products of the mutated allele do not show internalisation, which is required for ^{125}I to exert its effect on tumour cell nucleus [26].

In future molecular studies will allow the choice of target for adjuvant radioimmunotherapy of gliomas on an individual basis [22, 27]. Several new antigens have been considered as targets for labelled monoclonal antibodies in these malignant brain tumours, among them cancer-testis antigens [27]. Thus, we propose to continue the studies on the efficacy of adjuvant anti-EGRF ^{125}I radioimmunotherapy in a selected group of patients in whom the greatest benefit may be expected on the basis of combined expression profiling and immunohistochemical investigation.

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