Ratio of trastuzumab levels in serum and cerebrospinal fluid is altered in HER2-positive breast cancer patients with brain metastases and impairment of blood–brain barrier

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Patients receiving trastuzumab for HER2-overexpressing metastatic breast cancer seem to suffer from an increased risk of brain metastases, even in cases with responsive disease. To evaluate whether trastuzumab is able to penetrate the blood–brain barrier, we measured trastuzumab levels in the serum and in cerebrospinal fluid of metastatic breast cancer patients with brain metastases receiving trastuzumab for HER2-overexpressing metastatic breast cancer. In a pilot study, metastatic breast cancer patients with brain metastases and HER2-overexpressing tumors (HercepTest; Dako, Copenhagen, Denmark) were included. At different time points, trastuzumab levels in the serum and cerebrospinal fluid were measured using a newly developed immunoenzymatic test for trastuzumab. Six out of eight patients were evaluable for determination of trastuzumab level in the serum and cerebrospinal fluid. Before radiotherapy, median trastuzumab level in the serum was 52 054 ng/ml compared with 124 ng/ml in cerebrospinal fluid (ratio 420 : 1). After completion of radiotherapy, median trastuzumab level was 20 185 ng/ml in the serum and 226 ng/ml in cerebrospinal fluid, respectively (ratio 76 : 1). With concomitant meningeal carcinomatosis, trastuzumab level in the serum after radiotherapy was 17 431 and 356 ng/ml in cerebrospinal fluid (ratio 49 : 1). For the first time, we present clinical evidence that trastuzumab levels in cerebrospinal fluid are increased under conditions of an impaired blood–brain barrier such as meningeal carcinomatosis or radiotherapy. This evidence supports the concept of continuing trastuzumab therapy in patients with brain metastases treated by radiotherapy. Monitoring of trastuzumab levels in the serum and cerebrospinal fluid may enable individualized therapy strategies in metastatic breast cancer patients with brain metastases, and lead to a better understanding of trastuzumab pharmacokinetics in the cerebrospinal fluid and serum. *Anti-Cancer Drugs* 18:23–28 © 2007 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2007, **18**:23–28

Keywords: brain metastasis, cerebrospinal fluid, functional test, HER2-overexpression, metastatic breast cancer, trastuzumab

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Sponsorship: This research was supported in part by a grant from the Wilhelm-Sander Foundation (No. 2000.017.2) to H.B. and N.N. Roche, Mannheim, Germany, provided an educational grant to H.J.S. and M.S. to financially support this research.

Received 10 July 2006 **Revised form accepted** 7 August 2006

Introduction

Trastuzumab (Herceptin; Roche Pharma, Grenzach-Wyhlen, Germany) is a humanized monoclonal antibody directed against the HER-2/*neu* (c-erbB-2) oncoprotein. This cell membrane protein is encoded by the HER-2/*neu* gene and was characterized as a transmembrane growth factor receptor belonging to the epidermal growth factor receptor family, and is overexpressed in approximately 25–30% of all human breast carcinomas [1]. Preclinical and clinical studies have shown the ability of trastuzumab to inhibit tumor growth in patients with HER2-overexpressing breast cancer [1]. First-line use of trastuzumab together with chemotherapy significantly increases the response rate as well as the median overall survival in metastatic breast cancer (MBC) patients compared with chemotherapy alone [2].

Although brain metastases (BM) are clinically diagnosed in approximately 6–16% of MBC patients, several investigators reported an incidence of BM ranging from 25 to 48% in patients treated with trastuzumab-based regimen for HER2-positive MBC [3–13]. One possible explanation is that trastuzumab prolongs survival to such an extent that BM, which are known to be a late event in the course of metastatic disease, become apparent. The apparent change in the pattern of metastasis in HER2-positive breast cancer may thus be (partially) interpreted as a consequence of prolonged survival owing to trastuzumab treatment.

Little is known, so far, about the pharmacokinetics and state of activity of trastuzumab in the central nervous system. Yet, there is some evidence that trastuzumab may

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be unable to infiltrate into cerebrospinal fluid (CSF) [14,15]. In the scientific literature, only one single case has been reported showing that the concentration of trastuzumab was 300-fold lower in CSF than in serum [14,15]. Baculi *et al.* [16] reported on a breast cancer patient who presented with meningeal carcinomatosis responding to trastuzumab treatment and for whom impairment of the blood–brain barrier (BBB) was therefore suspected. Furthermore, Laufman and Forsthoefel [17] reported on a patient with meningeal carcinomatosis who was also treated successfully by intrathecal trastuzumab using an Ommaya reservoir.

The intact BBB limits the central nervous system penetration to molecules with molecular weights up to 200 Da (trastuzumab 185 kDa) [18]. Changes in the permeability of BBB were, however, observed in experimental animals as well as in clinical studies evaluating patients who received whole-brain radiotherapy (WBRT) for several malignant brain tumors [19].

For this report, a new immunoenzymometric test to assess the functional state of trastuzumab in the serum and CSF of breast cancer patients was developed. Using this highly sensitive test, we quantified reactive trastuzumab levels in serum and CSF of patients undergoing systemic treatment of MBC with trastuzumab, and correlated these values with histomorphological characteristics of the primary tumor and clinical features such as therapy details, radiotherapy (RT) or radiosurgery.

Patients and methods

Patient recruitment, trastuzumab-based treatment and response to therapy

Eight patients with MBC were recruited between August 2004 and March 2005 into a study approved by the Ethics Committee of the Medical Faculty of the Ludwig-Maximilians University, Munich, Germany. Prior to study entry, all patients gave written informed consent. All patients suffered from visceral metastatic disease and all presented with HER2-overexpressing primary tumors (score 3+ as assessed by immunohistochemistry applying the HercepTest; Dako, Copenhagen, Denmark). Trastuzumab was administered according to the accepted standard regimens, either weekly or triweekly with a loading dose of 4 mg/kg (8 mg/kg if every 3 weeks) followed by weekly doses of 2 mg/kg (6 mg/kg if every 3 weeks) either as single-agent therapy or in combination with the 5-fluorouracil pro-drug capecitabine (Xeloda; Roche Pharma; 1250 mg/m^2 twice daily orally, days 1–14, every 3 weeks). The clinical course, including assessment of metastatic sites and best response to therapy, was recorded on an intent-to-treat basis. Patient response to therapy was assessed using standard World Health Organization criteria. Clinically symptomatic CNS disease was confirmed by computed tomography or magnetic resonance imaging scan. Reactive trastuzumab levels in CSF were determined in CSF obtained by lumbal puncture. Meningeal carcinomatosis was diagnosed by CSF cytology using histological and immunocytochemical staining. Serum and CSF samples were shock-frozen immediately after acquisition and stored at −80°C until quantitative determination of reactive trastuzumab in these samples.

Determination of functionally active trastuzumab in serum and cerebrospinal fluid by enzyme-linked immunosorbent assay

The design of the functional trastuzumab enzyme-linked immunosorbent assay makes use of the interaction of trastuzumab with the extracellular domain (ECD) of HER2 and detection of this complex by an antibody to human IgG. For this, a 96-well microtiter plate (Maxisorp; Nunc, Wiesbaden, Germany) was coated with recombinant ECD of HER2. To construct a standard curve, sera from healthy female donors were spiked with different concentrations of trastuzumab (0–200 ng/ml) and then added to the ECD-coated wells of the microtiter plate. Likewise, sera or CSF from breast cancer patients were applied. As detecting antibody, alkaline phosphatase-conjugated mouse antibody to human IgG (Dianova, Hamburg, Germany) was employed. The amount of reactive trastuzumab was determined after addition of *p*-nitrophenyl phosphate; absorption of the color developed was measured at 405 nm in a multiwell-enzyme-linked immunosorbent assay reader (Spectra II; SLT Instruments, Crailsheim, Germany).

Results

Trastuzumab treatment and clinical course of metastatic breast cancer

Complying with the inclusion criteria, all MBC patients enrolled in this study suffered from BM and their primary tumor tissues showed HER2 overexpression that scored 3+ by immunohistochemistry applying the DAKO-HercepTest. Median interval from primary diagnosis of breast cancer to BM diagnosis was 5.72 years (range 0–10.5 years). Median survival after first diagnosis of MBC was 9.3 months (range 7.1–97.9 months) and median survival after first diagnosis of BM was 7.5 months (range 2.4–29.4 months). For detailed characteristics of the study population, see Tables 1 and 2.

Treatment for BM consisted of neurosurgery ($n = 3$), radiosurgery ($n = 2$) or WBRT ($n = 7$), or combinations of these modalities. Three patients presented with singular BM and six patients with multiple BM. Two patients were afflicted with meningeal carcinomatosis. One patient responded with partial remission to a trastuzumab-based regimen; six patients achieved stable disease during the time of observation. There were two patients

Table 1 Patient characteristics

Characteristics	*N*
Patients	8
Median age (range) (years)	59 (36–76)
Patients with immunohistochemistry score of 3+[a]	8
Steroid hormone receptor status (primary tumor)[b]	
ER or PgR positive	3
ER and PgR negative	5
Nuclear grade (primary tumor)[c]	
G1	0
G2	1
G3	7
Site of metastases[d]	
liver	5
lung	3
bone	3
soft tissue (lymph nodes)	2
BM	8
Appearance of BM[d]	
singular	3
multiple	6
meningeal carcinomatosis	2
Treatment of BM[d]	
whole-brain radiotherapy	7
radiosurgery	2
surgery	3

ER, estrogen receptor; PgR, progesterone receptor; BM, brain metastases.
[a]Determined by the HercepTest.
[b]Determined in primary tissue by immunohistochemistry.
[c]Determined in primary tissue.
[d]Some patients are mentioned twice owing to the combination of metastatic sites (sum >100%).

Table 2 Treatment, clinical course and patient survival

Characteristics	Median time intervals[a] (years/months)
Survival	
from first diagnosis of breast cancer	6.34 years
from first diagnosis of breast cancer to brain metastases	5.72 years
from first diagnosis of metastatic breast cancer	9.3 months
from first diagnosis of brain metastases	7.5 months
	N
Trastuzumab-based therapy	
single agent	4
in combination with capecitabine	4
Trastuzumab schedule (maintenance therapy)	
every 3 weeks (2 mg/kg body weight)	6
every 3 weeks (6 mg/kg body weight)	2
Systemic, visceral response to trastuzumab	
complete response	0
partial response	1
stable disease	6
progressive disease	0
not evaluable	1
Response to radiotherapy (WBRT/RS)	
complete response	2
partial response	1
stable disease	3
progressive disease	2
Cause of death (five patients alive)	
primarily of cns progression	1
systemic disease progression	2

WBRT/RS, whole-brain radiotherapy/radiosurgery; CNS, central nervous system.
[a]Five censored cases.

with complete and one with partial remission of BM owing to additional RT. Three patients reached stable disease; two progressed during WBRT.

Levels of functionally active trastuzumab in serum and cerebrospinal fluid of metastatic breast cancer patients before and after radiotherapy

We developed an immunoenzymometric test for detection of functionally active trastuzumab in body fluids, which is based on the interaction of trastuzumab with the immobilized ECD of HER2 and its detection by an enzyme-labeled antibody to human IgG (for details, see Patients and methods). This assay has a detection limit of 10 ng/ml. No functionally active trastuzumab was detected in the sera of female volunteers who had not received trastuzumab treatment. In all of the serum and CSF samples taken from the study MBC patients and analyzed by this assay, functionally active trastuzumab was detected, even though we observed a marked difference between the high trastuzumab levels in serum and the rather low levels present in CSF (Tables 3 and 4).

For three patients (case nos 1, 4 and 8) on long-term trastuzumab therapy (median 365 days), serum and CSF samples were collected at different time points and reactive trastuzumab was determined. The levels of reactive trastuzumab determined in serum at time points day 301 (no. 8), 365 (no. 1), and 996 (no. 4) ranged between 36 324 and 76 569 ng/ml, compared with levels between 112 and 168 ng/ml in CSF. Thus, the median trastuzumab level in CSF was 420-fold lower than the median serum level (Table 4).

Reactive trastuzumab levels determined in CSF after completing RT were available for five cases (nos 2, 4, 5, 7 and 8). Patients had been on trastuzumab between 35 and 1111 days (median 162 days) before serum and CSF samples were taken, and trastuzumab concentrations were determined. The median level of 266 ng/ml trastuzumab in CSF was 76-fold lower than a median reactive trastuzumab serum level of 20 158 ng/ml (Table 4).

Levels of functionally active trastuzumab in serum and cerebrospinal fluid of a metastatic breast cancer patient after neurosurgery

One patient (no. 5) with singular BM developed a cystic cerebral lesion after neurosurgical resection and WBRT. Furthermore, the patient developed meningeal carcinomatosis (118/3 cells) and sensory aphasia. As a consequence of these symptoms, the lesion was decompressed by stereotactic puncture on day 51 after completion of WBRT. The level of functionally active trastuzumab in the cyst fluid was rather high (16 371 ng/ml) compared with corresponding serum levels of 17 413 and 102 042 ng/ml and CSF levels of 998 and 682 ng/ml analyzed on days 6 and 97 after completion of WBRT.

Levels of functionally active trastuzumab in serum and cerebrospinal fluid of metastatic breast cancer patients with meningeal carcinomatosis

Levels of functionally active trastuzumab were determined in serum and CSF of two patients (case nos 5 and 7)

Table 3 Levels of functional, reactive trastuzumab in serum and CSF

Patient	RT/RS	CSF cytology	Days on trastuzumab	Reactive trastuzumab in serum (ng/ml)	Reactive trastuzumab in CSF (ng/ml)	Days after RT/RS	Days on trastuzumab	Cytology	Reactive trastuzumab in serum (ng/ml)	Reactive trastuzumab in CSF (ng/ml)
1[a]	WBRT	0	365	36 324	124	–	–	–	–	–
2	WBRT/ RS	–	–	–	–	152	456	3	203469	158
						198	502	2	77404	193
3[a]	WBRT	14	1	1	5	–	–	–	–	–
4	RS	1	996	52 054	112	99	1111	4	16283	32
5[b]	WBRT	21	1	3	1	6	44	118	17431	998
						16	54	–	–	16 371[c]
						97	135	9	102042	682
6[a]	WBRT	1	1	10	1	–	–	–	–	–
7[b]	WBRT	–	–	–	–	24	0	9	4	33
						59	35	77	16304	258
						164	140	12	22885	356
8	WBRT	2	301	76 569	168	13	333	7	15474	273

WBRT, whole brain radiotherapy; RT, radiotherapy; RS, radiosurgery; CSF, cerebrospinal fluid.
[a]Patient died before completing RT.
[b]Patient with meningeal carcinomatosis.
[c]Patient with a cystic brain metastasis after stereotactic puncture.

Table 4 Levels of functional, reactive trastuzumab in serum and CSF of MBC patients in relation to clinical parameters and therapy details

Patient	Days on trastuzumab	Days after RT	Meningeal carcinomatosis	Reactive trastuzumab in serum (ng/ml)	Reactive trastuzumab in CSF (ng/ml)	Ratio trastuzumab serum : CSF
Occurrence of trastuzumab in serum and CSF						
1	365	(before RT)	-	36324	124	
4	996	(before RT)	-	52054	112	
8	301	(before RT)	-	76569	168	
median	**365**			**52054**	**124**	**420**
Occurrence of trastuzumab in serum and CSF after RT						
2	456	152	-	203469	158	
	502	198		77404	193	
4	1111	99	-	16283	32	
5	44	6	+	17431	998	
	135	97		102042	682	
7	35	59	+	16304	258	
	140	164		22885	356	
8	333	13	-	15474	273	
median	**162**	**98**		**20158**	**266**	**76**
Occurrence of trastuzumab in serum and CSF for patients after RT and with meningeal carcinomatosis						
5	44	6	+118/3 cells	17431	998	
7	35	59	+77/3 cells	16304	258	
	140	164	+12/3 cells	22885	356	
median	**44**	**59**		**17431**	**356**	**49**

CSF, cerebrospinal fluid; RT, radiotherapy (radiosurgery, whole-brain radiotherapy).

suffering from meningeal carcinomatosis. Both patients had received WBRT for intracerebral metastases. Patients had been on trastuzumab for 35 and 140 days, respectively, before serum and CSF samples were collected. One patient (no. 7) received intermittent corticosteroid treatment for symptoms caused by meningeal carcinomatosis. The median level of reactive trastuzumab in CSF of 356 ng/ml (range: 258–998 ng/ml) was 49-fold lower than the median trastuzumab serum level in these patients (median 17 431 ng/ml; range: 16304–22885 ng/ml).

Discussion

The humanized antibody trastuzumab (Herceptin) directed to the plasma membrane-associated oncoprotein HER2 is an effective treatment for patients with MBC overexpressing HER2. Yet, an unexpectedly high incidence of BM has been observed in MBC patients receiving trastuzumab therapy. This led to the assumption that trastuzumab therapy may change the disease pattern in HER2-positive MBC by prolonging survival or that metastasis in these patients may have a preference for the brain [3–13]. In HER2-positive MBC, the BBB may prevent trastuzumab from reaching adequate concentrations in CSF, thus making development of BM more common even in case of trastuzumab-sensitive metastatic disease [9–11,14,15].

Little is known so far about the pharmacokinetics of trastuzumab in CSF as compared with the circulating blood compartment. To further evaluate this increasingly important clinical problem, we developed and applied a

new test system to quantitatively measure functionally active trastuzumab in serum and CSF of MBC patients with BM, whose primary tumors overexpress the oncoportein HER2 and who received first-line trastuzumab therapy for advanced disease. At present, there is only a single report in the literature showing that in an MBC patient with BM, trastuzumab concentrations were 300-fold lower in CSF than in serum [14,15]. Our own results confirm these findings by Pestalozzi and Brignoli [14]. In our study, trastuzumab levels in CSF of patients before RT were about 420-fold lower than in serum. One could argue that trastuzumab may need more time to achieve a steady-state rate in CSF. All three patients had, however, received trastuzumab for more than 300 days.

Whether intravenous trastuzumab (molecular weight 185 kDa) can reach the CSF or penetrate the brain tissue after RT of BM and whether therapeutically effective levels of trastuzumab in CSF can be achieved in patients with impaired BBB has not been evaluated so far. Some evidence, however, exists that permeability of the BBB is increased in patients who received RT for malignant brain tumors [19]. Therefore, one aim of our study was to evaluate whether RT (WBRT or radiosurgery) will lead to the impairment of the BBB and subsequent penetration of trastuzumab into CSF. Generally, the intact BBB limits the central nervous system penetration to molecules with molecular weights up to 200 Da [18].

After completion of RT, we found a median CSF level of reactive trastuzumab of 266 ng/ml compared with a median serum level of 20 158 ng/ml in our MBC patients with standard intravenous trastzumab therapy. Hence, after RT for BM and a median duration of 162 days on trastuzumab, the median trastuzumab level in CSF was only 76-fold lower than that in serum. This corresponds to a substantial improvement compared with the trastuzumab serum/CSF ratio of 420 : 1 in patients without RT for BM. The new test system, as described here, provides the basis for evaluation of radiotherapeutic strategies that allow the most favorable penetration of trastuzumab into the CNS of patients with BM receiving concomitant trastuzumab for HER2-overexpressing MBC.

Baculi *et al.* [16] observed a single MBC patient with meningeal carcinomatosis who responded to trastuzumab treatment and for whom, consequently, disintegrity of the BBB was suspected. In our study, reactive trastuzumab CSF levels were determined in two of our patients with cytologically confirmed meningeal carcinomatosis and then compared with the corresponding serum levels. Although patients had only been on trastuzumab therapy for a relatively short period (median 440 days), their median trastuzumab serum level was 17 431 ng/ml and thereby only 49 times higher than the corresponding median CSF level (356 ng/ml).

In summary, the serum/CSF trastuzumab ratio of 420 : 1 determined in patients before RT is substantially lower under conditions of impairment of the BBB with a ratio of 76 : 1 in patients after RT and of 49 : 1 in patients severely affected with meningeal carcinomatosis. For the latter patients with HER2-overexpressing meningeal carcinomatosis, intrathecal therapy with trastuzumab seems to be a promising approach, which should be further investigated [20].

For the first time, we present evidence that the humanized antibody to HER2 trastuzumab only reaches rather low and most likely therapeutically inadequate levels in CSF after intravenous application but that penetration of trastuzumab into CSF is facilitated under conditions of an impaired BBB such as meningeal carcinomatosis or BM RT. Our results support the concept of continuing trastuzumab therapy in patients with BM and responsive metastasis outside the CNS.

An increasing use of trastuzumab for therapy of HER2-overexpressing breast cancer was seen in the advanced and in the adjuvant setting. Thus, more patients with HER2-positive tumors are expected to derive a substantial survival advantage compared with similar patients treated when trastuzumab was not available. Yet, these patients are at considerable risk for experiencing progressive disease to the CNS during or subsequent to trastuzumab treatment. For such patients, with CNS metastasis, the development of new treatment strategies on the basis of the monitoring of reactive trastuzumab level in serum and CSF are quite feasible. Moreover, validation of our results may also lead to a better understanding of trastuzumab pharmacokinetics in CSF and serum, and enable strategies for prevention of BM in trastuzumab-treated breast cancer patients.

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