Does gamma knife surgery stimulate cellular immune response to metastatic brain tumors? A histopathological and immunohistochemical study

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Object. The aim of this study was to analyze the cellular immune response and histopathological changes in secondary brain tumors after gamma knife surgery (GKS).

Methods. Two hundred ten patients with cerebral metastases underwent GKS. Seven patients underwent subsequent craniotomy for tumor removal between 1 and 33 months after GKS. Four of these patients had one tumor, two patients had two tumors, and one patient had three. Histological and immunohistochemical investigations were performed. In addition to routine H & E and Mallory trichrome staining, immunohistochemical reactions were conducted to characterize the phenotypic nature of the cell population contributing to the tissue immune response to neoplastic deposits after radiosurgery. Light microscopy revealed an intensive lymphocytic infiltration in the parenchyma and stroma of tumor samples obtained in patients in whom surgery was performed over 6 months after GKS. Contrary to this, extensive areas of tissue necrosis with either an absent or scanty lymphoid population were observed in the poorly controlled neoplastic specimens obtained in cases in which surgery was undertaken in patients less than 6 months after GKS. Immunohistochemical characterization demonstrated the predominance of CD3-positive T cells in the lymphoid infiltration.

Conclusions. Histopathological findings of the present study are consistent with a cellular immune response of natural killer cells against metastatic brain tumors, presumably stimulated by the ionizing energy of focused radiation.

Key Words • gamma knife surgery • metastases • T-cell lymphocyte

Brain metastases develop in up to 30% of patients suffering from malignant disease,23 and almost one half of them originate from lung carcinoma.1 The natural course of untreated patients harboring brain metastases results in a median survival of 1 month.11,24 Fractionated radiation therapy has been shown to prolong survival to 3 to 6 months in large series.7,12 Excision combined with radiotherapy improves the outcome compared with radiation therapy alone;20,21,27 however, surgery is only used in patients in good medical condition and is generally recommended for solitary, accessible tumors. Because metastatic brain tumors are usually well-delineated and circumscribed lesions, they are suitable targets for radiosurgery. Recent publications have demonstrated that stereotactic radiosurgery is an effective method for the treatment of secondary brain tumors irrespective of the histological subtype, including traditionally radioresistant lesions, multiple tumors, and recurrent tumors.1,4,6,10,14,19,22 Complications following stereotactic radiosurgery are uncommon, and the risk for permanent neurological dysfunction is low.8 Although the treatment strategy is well established for secondary brain tumors, our knowledge of the biological and pathophysiological mechanisms resulting in tumor control or failed treatment after stereotactic radiosurgery is limited.4 Therefore, the goal of this study was to investigate histopathological changes in cerebral metastases that had been treated with GKS as a first-line intervention and later removed by craniotomy due to failure of local control. Factors influencing treatment outcome and the possible contribution of the cellular immune system in tumor destruction following GKS are discussed.

Clinical Material and Methods

Seven of 210 patients treated with GKS for cerebral metastases underwent subsequent craniotomy for tumor removal, because the treated lesion showed further growth on follow-up imaging. Four of these patients had one tumor, two patients had two tumors, and one patient had three. The primary tumor was breast carcinoma in three patients, lung cancer in two, and malignant melanoma in two. Gamma knife surgery was performed using the Leksell Gamma Knife model C (Elekta Instruments AB, Stockholm, Sweden). Dose planning was based on magnetic resonance and computerized tomography imaging; metabolic data of positron emission tomography studies was also integrated in selected cases.17,18 Treated volumes ranged between 35 and

Abbreviation used in this paper: GKS = gamma knife surgery.
8090 mm$^3$; the margin dose ranged from 16 to 24 Gy; the prescription isodose varied between 48 and 50%, and the maximum dose ranged from 32 to 48 Gy. Because of neurological and radiological progression, the lesions were removed at craniotomy. The interval from GKS to surgery ranged from 1 to 33 months.

Histopathological investigations were performed on the tissue gathered at operation. The resected specimens were fixed in 10% neutral buffered formaldehyde, processed routinely, and embedded in paraffin. In addition to routine H & E and Masson trichrome staining, immunohistochemical reactions were conducted for CLA, CD3, CD4, UCHT1, CD8, CD79a, and L26 (CD20) antigens to characterize the phenotypic nature of the lymphoid cell population infiltrating the neoplastic tissues. Biotin-streptavidin-peroxidase complex methods were performed according to standard protocols on 5-$\mu$m sections. The monoclonal antibodies used in this study were provided from DAKO A/S, Denmark. Five surgically treated cases involving different histological types of cerebral metastases and without previous radiotherapy or radiosurgery served as nonirradiated controls.

Results

Histopathological Findings

Light microscopic investigations of the surgical specimens demonstrated histological changes attributable to radiosurgery, which were the probable result of the high-dose radiation. Two basic types of tissue reactions were explored. One was associated with a better tumor control after radiosurgery, in that the interval from GKS to surgery was over 6 months. In the five patients in whom this applied, sharply demarcated areas of coagulation necrosis were surrounded by differing amounts of granulation tissue together with small vessels (that is, arterioles, capillaries, and venules). The granulation tissue reaction was accompanied by a striking inflammatory cell infiltration, which varied across the volume of the GKS-induced lesions. The necrotic center of the lesions consisted of tissue debris mixed with fibrinoid deposits, shrunken pyknotic apoptotic cells and polymorphonuclear leukocytes as illustrated in Fig. 1A. A transitional zone containing mainly macrophages incorporating tumor cells, cellular debris, and red blood cells was situated around the necrotic.

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**Fig. 1.** A: Sharply demarcated coagulation necrosis with neutrophile leukocytes in the center of a metastatic carcinoma 17 months following GKS. B: Inflammatory cell infiltration at the periphery of the necrotic region consisting of macrophages and plasma cells. C: Hyaline degenerated scar tissue replacing destroyed tumor cell nests 3 years after GKS. D: Postirradiation vascular changes in the connective tissue stroma of a metastatic tumor: spindle-cell proliferation in the subendothelial region and vessels’ wall 17 months after GKS. H & E, original magnification $\times$ 200.

**Fig. 2.** A: Intense lymphocytic infiltration in the capsule zone of a metastatic melanoma 1 year after GKS. B: Lymphoid infiltration propagates into the tumor parenchyma as well. C: Lymphocytic infiltration originates from a perivascular space of the remaining normal brain parenchyma 17 months after GKS. D: Lymphoid infiltration becomes more pronounced toward metastatic nests. E: Granulation tissue rich in postcapillary venules infiltrated by lymphocytes 17 months after GKS. F: Prominent cellular atypia, nuclear fragmentation, and apoptotic bodies at the region of lymphoid invasion. H & E, original magnification (A, B, D) $\times$ 100 and (C and E) $\times$ 200. Inset (F) $\times$ 400.
core as shown in Fig. 1B. The presence of both hemosiderin and fresh red blood cells suggested repeated intratumoral bleeding, which could cause an increase in the volume of a lesion. Hyaline-degenerated hypocellular scar tissue seemed to replace the necrotic areas at later stages after GKS (Fig. 1C). Postirradiation vascular changes in the connective tissue stroma were usually present in the form of subendothelial cell proliferation (Fig. 1D). The most striking and common histopathological feature of this well-controlled group was an intensive and extensive, dense lymphocytic infiltration of the tumors’ connective tissue stroma (Fig. 2A). The tissue also formed nests within the tumor parenchyma itself (Fig. 2B). This lymphoid infiltration seemed to originate from the perivascular spaces of adjacent normal brain tissue (Fig. 2C) and it became more pronounced toward the tumor cell nests (Fig. 2D). The lymphoid infiltration was accompanied by a granulation tissue rich in postcapillary venules (Fig. 2E). Where the lymphoid infiltration encroached on residual nests of tumor cells, cellular atypia, nuclear fragmentation, and formation of apoptotic bodies were evident (Fig. 2F).

The findings were different in the two patients with poorly controlled tumors surgically treated at less than 6 months after GKS. The findings were similar to nonirradiated regions of metastatic tissue. There were large sheets of neoplastic tissues alternating with huge necrotic areas but with an absent or scant lymphoid cell population (Figs. 3A and B).

**Immunohistochemical Results**

The most remarkable immunohistochemical finding was the marked positivity of the CD3 reaction on the lymphoid elements in the well-controlled tumor specimens. This reaction demonstrated the presence of CD3-positive T lymphocytes in large numbers (Fig. 4 left). These cells correspond to natural killer cells and reflect a cellular immune reaction against metastatic tumor cells; however, these cells were basically absent or just sporadic in the poorly controlled neoplastic tissue samples and nonirradiated control tissue (Fig. 4 right). This finding suggests a lack or impairment of cellular immunity in these cases.

**Discussion**

Radiosurgery represents a treatment modality that is increasingly important in the management of cerebral metastases. Although nearly 100,000 GKS procedures have already been performed worldwide to treat cerebral metastases, the pathophysiological mechanisms involved are still far from completely understood. The processes leading to destruction of a lesion or a failed treatment outcome have yet to be determined.

Apart from early basic investigations, most available pathological data of radiation-induced changes in human brain tissue have been derived from poorly documented autopsy cases, or they have been collected from papers involving the use of conventional wide-field radiotherapy. Rapid development and refine-
Cellular immune response after GKS

ment of neuroimaging techniques during the past three decades and the precise isodose-related radiation delivery of GKS permit the sophisticated pathological analysis of post-irradiation changes in the targeted volume. Such studies would promote better understanding of the pathophysiological mechanisms leading to radiation-induced alterations, and could provide important information which could permit better future treatment planning for similar lesions.16

The goal of radiosurgery in the management of single or multiple cerebral metastases is total tumor destruction, or at least control of the proliferative process otherwise called local tumor control. According to current theories, local tumor control might be achieved through different pathophysiological processes such as apoptosis, vascular damage, and coagulation necrosis. Radiosurgery may destroy or control the targeted necrotic cell proliferation either by direct early cytotoxic effects (coagulation necrosis or apoptosis) or by late vascular changes. Kondziolka et al.15 suggested that the radiobiological mechanism of radiosurgery on benign tumors is a combination of both cytotoxic and vascular effects. The cytotoxic effect of radiation on DNA may not be apparent at once but may be seen after subsequent cell divisions with cell death arising during a subsequent cell cycle via the mechanism of apoptosis or lysosomal-induced necrosis. Rapidly proliferating tumors with a high cell division rate, such as metastases or malignant gliomas, react earlier to irradiation than slowly growing benign tumors. Lesions without spontaneous proliferating activity like arteriovenous malformations also react with a prolonged latency period. The late vascular effect of radiosurgery appears through modification of wall structures in the lesions’ channels. Radiosurgical therapeutic doses usually do not affect normal brain vessels; however, it seems that the pathological vessels of vascular malformations have a relative sensitivity to high-dose irradiation compared with normal surrounding or feeding arteries.11,12,13,24,25,26

Histopathological investigations in the present study revealed both necrotic and apoptotic changes in the neoplastic parenchyma, and typical postirradiation structural modifications in vessel walls leading to subtotal or even total obliteration of vessels in the of tumors’ connective tissue stroma. These tissue alterations might be evoked by the ionizing energy of high-dose irradiation and were anticipated from previous histopathological studies.11,12,13 The most surprising and unexpected findings, however, were presented by the immunohistochemical reactions. These studies showed the presence of an intensive lymphocytic infiltration in the relatively well-controlled tumor group after radiosurgery, which was absent or negligible in the less well-controlled neoplasms and in nonirradiated control cases.

Antigen characterization revealed a predominance of CD3-positive T lymphocytes, that is, natural killer cells in the infiltrating lymphoid population. This is consistent with a cellular immune response against metastatic tumor cells. These findings seem to be in agreement with the results of recent well-controlled animal experiments in which the absence of natural killer cell–mediated defenses was suggested in nonirradiated mouse brain tumors.16 The stimulation of cellular immune reactivity by GKS may act in one of two ways. First, radiation could induce an impairment of the blood–brain barrier, which could facilitate the escape of white blood cells from vessels to the extravascular compartments. This theory is supported by the histopathological finding that the lymphoid infiltration seemed to begin and spread via the perivascular spaces of the brain. Second, the well-known sterile inflammatory cellular reaction evoked by radiosurgery in other pathological conditions like arteriovenous malformations may contribute as well.15,24,26 A similar stimulation of the immune system is suspected to underlie the so-called abscopal phenomenon, in which remote effects produced by radiation occur in regions away from high radiation dose.9

The histopathological and immunohistochemical findings of the present study suggest that after GKS a vigorous cellular immune reactivity seems to occur in patients in whom long-term tumor control occurs, whereas there seems to be a degree of impaired T lymphocyte function in cases in which tumor control is relatively poor. Stimulation of a cellular immune response could be attributed to the ionizing effect of focused irradiation.

Conclusions

After GKS long-term brain metastasis control seems to be associated with an intensive lymphocytic infiltration. This lymphocytic reaction was absent or negligible in poorly controlled cases. A predominance of CD3-positive T cells was identified in the aforementioned lymphoid infiltration. These are killer T cells. Thus it seems that a cellular immune response against metastatic neoplastic cells may be induced by radiosurgery.

References

14. KIM YS, KONDZIOLKA D, Flickinger JC, et al: Stereotactic radio-

G. T. Szeifert, et al

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