L'expérience radiochirurgicale

Résultats

HISTOPATHOLOGICAL OBSERVATIONS ON VESTIBULAR SCHWANNOMAS AFTER GAMMA KNIFE RADIOSURGERY: THE MARSEILLE EXPERIENCE

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SUMMARY: Histopathological observations on vestibular schwannomas after Gamma Knife radiosurgery: the Marseille experience


Background and purpose. — Radiosurgery has become a successful treatment modality in the management of vestibular schwannomas (VS) during the past four decades. Although the number of treated cases has been increasing continuously we know relatively little about the pathological effect of high dose irradiation on VS following radiosurgery. The purpose of this study was to analyze histopathological changes in VS after Leksell Gamma Knife (LGK) radiosurgery.

Methods. — Out of a series of 1350 VS cases treated with LGK surgery 22 patients underwent craniotomy for tumor removal in 6-92 months interval after radiosurgery. Surgical pathology material was available in 17 cases. Routine histological and immunohistochemical investigations were performed on the tissue samples. Histopathological findings were compared with clinical and radiological follow-up data.

Results. — Coagulation necrosis in the central part of the schwannomas surrounded with a transitional zone containing loosened tissue structure of shrunken tumor cells covered with an outer capsule of vigorous neoplastic cells was the basic histopathological lesion. Granulation tissue proliferation with inflammatory cell infiltration, different extent of hemorrhages and scar tissue development was usually present. Endothelial destruction or wall damage of vascular channels was a common finding. Analyzing the follow-up data it turned out that 7 patients out of the 22 were operated on because of radiological progression only without clinical deterioration and 4 of them was removed during the latency period after radiosurgery.

RÉSUMÉ Caractéristiques histopathologiques des schwannomes vestibulaires après radiochirurgie Gamma Knife : l’expérience marseillaise

La radiochirurgie est aujourd’hui considérée comme un traitement efficace des schwannomes vestibulaires (SV). En dépit du nombre sans cesse croissant de patients traités par cette technique, les effets de l’irradiation à haute dose unique sur le tissu tumoral schwannomateux in vivo sont peu connus. Cette étude a pour but d’étudier les modifications histopathologiques observées après radiochirurgie gamma knife (RGK) des SV. Sur un nombre total de 1 350 SV successivement traités par RGK à l’Hôpital de la Timone, 22 patients ont nécessité une exérèse chirurgicale dans un délai compris entre 6 et 92 mois après la radiochirurgie. Le tissu tumoral a pu être disponible pour étude pathologique et immunohistochimique complémentaire dans 17 cas. Les données de ces investigations ont été couplées aux renseignements cliniques et radiologiques des patients.

Les données histopathologiques régulièrement observées consistaient en une plage de nécrose de coagulation distribuée au centre de la tumeur, entourée d’une zone transitionnelle faite d’une trame tissulaire moins dense constituée de cellules tumorales tronquées, elle-même couverte d’une couche périphérique de cellules tumorales actives. On pouvait aussi fréquemment observer un infiltrat de cellules inflammatoires dans un tissu de granulation, des plages hémorragiques et des zones cicatricielles. De même, des zones de lésions des parois vasculaires, en particulier endothéliales, étaient souvent identifiables. Il faut noter que 9 tumeurs avaient été réséquées sur une augmentation volumétrique sans aggravation clinique, dont 4 pendant la phase de latence précoces après la radiochirurgie.

Les résultats de cette étude suggèrent que la radiochirurgie Gamma Knife agit sur le schwannome par un
Conclusion. — Results of the present histopathological study suggest that radiosurgery works with double effect on VS: it seems to destroy directly tumor cells (with necrosis or inducing apoptosis), and causes vascular damage as well. The loss of central contrast enhancement on CT and MR images following radiosurgery might be consequence of necrosis and vascular impairment. From clinical-pathological point of view we think that patients should not undergo craniotomy just because of radiological progression of the tumor without clinical deterioration, mainly in the latency period. This requires consultation and common decision-making between the radiosurgical and the microsurgical team.

Key-words: Gamma Knife radiosurgery, vestibular schwannoma, histopathology.

Since June 1968, when the first patient was treated in Stockholm with vestibular schwannoma by Professor Lars Leksell [14], stereotactic radiosurgery [12] has become an effective and successful treatment modality in the management of these neoplasms. Nowadays three main stereotactic radiosurgical techniques are available for this purpose: the Leksell Gamma Knife [3, 8, 16, 20, 21, 24, 25], modified linear accelerators [18, 19, 28] and proton or heavy-ion charged particles generated in cyclotron [17]. Currently radiosurgery may offer a sophisticated and safe alternative possibility to micro-neurosurgery in selected cases. On the other hand it can serve as a complementary tool for residual or recurrent VS cases after craniotomy related conventional surgery. Although the number of treated cases has been increasing continuously year to year, only sparse publications available in the literature deal with the pathological effect of radiosurgery on VS [6, 10, 31].

The goal of the present study was to analyze morphological changes in a series of 17 VS cases operated on by conventional craniotomy after Leksell Gamma Knife radiosurgery. Histopathological findings in the removed tumor samples were compared with treatment parameters, radiological imaging and neurological clinical follow-up data. The first author of the paper (GTS) had the exceptional chance to review this unique surgical pathology material in the World as an independent expert from another institution.

PATIENT, MATERIAL AND METHODS

Out of a series of 1350 VS cases treated with LGK radiosurgery since 1992 by the senior author (JR) in the Centre Gamma Knife, Université Hôpital La Timone Marseille, France, 22 patients underwent subsequent craniotomy for microsurgical tumor removal in different neurosurgical units. The age of the patients at the time of treatment ranged from 27 to 70 years, median 51 years. As a primary intervention they were treated with radiosurgery using the Leksell Gamma Knife® Model-C (Elekta Instruments, Stockholm, Sweden). The dose planning was based on MR and CT imaging with the Leksell Gamma Plan® software. The treated volume of the 22 microsurgery operated cases ranged between 168 and 11 405 mm³, median 1367 mm³. The tumors received 9-15 Gy, median 12 Gy as peripheral dose at the 50% isodose line, the maximum dose in the center ranged from 18-30 Gy, median 24 Gy. The number of shots varied between 3 and 28, median 7. The irradiated neoplasms were removed by conventional craniotomy related microsurgery in a second step because of simple radiological, or combined radiological and neurological progression after 6-92 months, median 35 months interval following radiosurgery. Surgical pathology material was available from 17 patients therefore the 5 other cases were excluded from the histopathological study.

Microscopical investigations were carried out on the 17 radiosurgically treated and microsurgery removed lesions. Five other VS operated cases without previous radiosurgical intervention served as non-irradiated controls. The resected specimens were fixed in 10% neutral buffered formaldehyde, processed routinely, and embedded in paraffin. Besides the conventional hematoxylin-eosin, orceine, Perls’ and Masson’s trichrome stainings immunohistochemical reactions were carried out for Ki67 antigen to scrutinize proliferative capacity of tumor cells. FVIII-related, CD31 and CD34 antigens were also studied to demonstrate endothelial activity and vascular effects of the irradiation on tumor vessels. Biotin-streptavidine-peroxidase complex methods were performed according to standard protocols on 5µm sections. The following antibodies were used in this study: anti-FVIII (Rabbit Anti-Human polyclonal, DAKO A/S, Denmark), anti-CD31 and anti-CD34 (monoclonal QBend/10, BioGenex, USA) to highlight the endothelial cell layer and smooth muscle wall of channels.
RESULTS

CLINICAL DATA

Out of the 22 patients, 7 were operated on because of radiological progression only without clinical deterioration, and the remaining 15 cases presented with combined neurological and imaging progression as well. In 6 patients, the initial state was Koos grade 2 at the time of LGK surgery and grade 3 at microsurgical resection. In 6 patients, the clinical condition changed from grade 3 to grade 4, and in 3 patients from grade 2 to grade 4. The remaining 7 did not change. The volume of the tumors varied between 866 and 19709 mm$^3$ (median: 5324 mm$^3$) at the time of microsurgical resection. In 15 cases, total removal was achieved; in 4 patients, a microfragment was left attached to the brainstem, and in the other cases subtotal resection was manageable. In 10 cases, the surgeon found some extra-difficulties during the operation; the remaining cases were removed routinely. In 3 cases, retrosigmoid exploration was selected; the others were removed via the translabirinth approach. Facial nerve function was postoperatively House-Brackman grade 1 in 6 cases, grade 2 in 4 cases, grade 3 in 7 cases, grade 4 in 2 cases, grade 5 in 2 cases, and follow-up data were not available in 1 case.

MORPHOLOGICAL FINDINGS

Histopathological changes affected both the parenchyma and the stroma of the radiosurgery treated VS tissue samples. The basic histopathological lesion was a necrotic core surrounded by a middle transitional zone covered by an outer capsule (figure 1a, b). The inner core of tumors consisted of necrotic debris containing scattered shrunken apoptotic neoplastic cells, with narrow cytoplasmic rim and dark pycnotic basophilic nuclei without recognizable nucleoli. Mostly only the contour of the tumor cell nests was suspected or just a homogeneous mass without any morphological details was found. There were no identifiable vascular elements in this zone. This histological picture was characteristic of a coagulation necrosis or ischemic infarction. This area was sharply demarcated from the surrounding tissues. According to a previous study this region gets the highest dose of irradiation beyond the 70% isodose line and constitutes the so-called “cystic” part of the tumor [28]. These pathological findings correlated with the radiological appearance demonstrating signal changes, hypodensity and lack of contrast enhancement in the inner part of tumor tissue on CT and MR images after radiosurgery.

The outer capsule or mantle zone of neoplasms usually presented densely packed tumorous Schwann cell nests with storiform pattern or nuclear palisade arrangement. At high magnification, vigorous tumor cells with abundant eosinophilic cytoplasm, large oval nuclei with or without nucleoli were seen in this region. Different degree of granulation tissue proliferation containing thin-walled capillaries and small vessels infiltrated by inflammatory cells, hemosiderin-laden macrophages and occasionally foamy cells was generally present (figure 2). Scar tissue formation among and around tumor cell nests was

FIG. 1. — The basic histopathological lesion in vestibular schwannoma after radiosurgery: sharply demarcated necrotic areas towards surrounding tissue (Masson’s trichrome, ×100). b) Outer capsule or mantle zone, middle transitional zone and inner necrotic core in the radiolesion (H&E, ×200).

FIG. 1. — a) Lésion histopathologique de base du schwannome vestibulaire après radiochirurgie. Zones de nécrose bien délimitée vers le tissu environnant (trichrome de Masson, ×100). b) Capsule externe ou zone de manteau, la zone médiane de transition, et la zone centrale de nécrose d’une radiolésion (HE ; ×200).
a common finding with thick collagen bundles, hyaline degeneration, proliferating fibroblasts and fibrocytes. As the time interval had increased from radiosurgery, this zone became more hypocellular and was replaced by bulky connective tissue (figure 3). However, living tumor tissue remnants were demonstrated in all of the 17 treated VS cases, even 92 months following radiosurgery as well. Most of the tumor specimens expressed dilated blood filled capillaries and veins with patchy hemorrhages. In two cases, extensive hemorrhagic areas destroyed surrounding tumor tissue. Vascular elements of tumors were injured by degenerative processes like endothelial destruction, vessel-wall damage and hyaline degeneration. In an NF2 patient with the shortest time interval (6 months) between radiosurgery and microsurgical removal foci of high proliferative activity were expressed. Areas of high cell density containing crowded vigorous tumor cell nests with cellular atypia, nuclear polymorphism, prominent nucleoli and frequently multinuclaeated giant cells were recognized (figure 4). The capsule area receives the lowest doses of irradiation during radiosurgery, usually at about the 50-60% isodose line.

The transitional zone of the tumors between the outer capsule and inner necrotic core at about the 60-70% isodose line demonstrated loosened tissue structure, and the cells became shrunken with more compact nuclei without nucleoli. This region was commonly infiltrated by granulation tissue as well.

In one case, a right sided VS was removed microsurgically 36 months after radiosurgery (figure 5a), and a glioblastoma was resected from the right temporal lobe 100 months after radiosurgery (figure 5b).

**IMMUNOHISTOCHEMICAL RESULTS**

Ki67 proliferative activity was explored in 10-20 cells per high power field in non-irradiated control VS specimens (figure 6a). Decreased activity was noticed 6 months after radiosurgery (figure 6b), and it was only occasional 16 months after radio-
surgery (figure 6c). Moderate but still existing proliferative capacity was seen even 48 months after radiosurgery as well (figure 6d).

Vigorous FVIII-related antigen positivity was expressed in the endothelial layer of the neoplastic vessels in the non-irradiated control VS samples (figure 7a). Diminished reaction was demonstrated in tumor vessels 6 months after radiosurgery (figure 7b). Modest positivity was noticed 16 months after radiosurgery (figure 7c), and returned strong activity was seen in the VS channels 48 months after GK treatment (figure 7d).

Marked CD31 positivity was revealed in endothelial cells of tumor vessels in non-irradiated control VS tissue specimens (figure 8a). This reaction decreased considerably 6 months following GK surgery (figure 8b), and basically no reaction could be identified 16 months after radiosurgery (figure 8c). However recurrent reactivity was detected 48 months following GK treatment (figure 8d).

Conspicuous CD34 positivity was demonstrated in the vessels’ wall from non-irradiated VS pieces (figure 9a). This reaction diminished 6 months after radiosurgery (figure 9b), and became faint 16 months following GK treatment (figure 9c). Striking CD34 reactivity was noticed again in the channels 48 months after GK treatment (figure 9d), and it still was prominent in the granulation tissue of a VS case even after 72 months from radiosurgery (figure 9e).

DISCUSSION

Stereotactic radiosurgery has become a successful and continuously developing treatment modality in the neurosurgical realm during the past three and half decades. Since the first patient was treated in January 1968 at the Sophiahemmet Hospital in Stockholm, Sweden, with the prototype Gamma Knife more than 200,000 patients have been operated on worldwide with this technique. Although the treatment indications and the number of treated patients has been increasing considerably, we know relatively little about the pathological background explaining radiobiology and pathophysiological mechanisms leading to cure or undesired side effects. According to Kondziolka et al. the future of radiosurgery among others will be built on the better understanding of the biological effect of radiation, which will enable treatment of new disorders [9].

In 1958, the first radiosurgical pathology landmark paper by Larsson, Leksell et al. demonstrated in animal experiments that “with high-energy protons a sharply delimited lesion can be made at any desired site in the central nervous system” [11]. The basic histopathological lesion created by high energy ionizing radiation in brain tissue is a coagulation necrosis within the target volume, which did not change in time, and the boundary between the necrosis and the surrounding normal structures is distinct, according to the sharp radiation fall-off [1, 13, 32].

Besides the experimental results a few reports have already been published on human pathological data concerning histological changes in cerebral arteriovenous malformations (AVM) after radiosurgery [26, 29, 30, 33, 34]. These papers have concluded that nidus occlusion in AVM is achieved through a thrombo-obliterative process in the vessels evoked by the ionizing energy of irradiation. In
a previous study from the Royal Hallamshire Hospital of Sheffield, England we have analyzed the histopathological changes in AVM after LGK radiosurgery [29, 30]. The conclusion of the histological analysis was that proliferation of a spindle-shaped cell population with contractile capacity, generated by the gamma-irradiation in the subendothelial region of the vessels' wall and stroma of the AVM could be relevant to the shrinking process and eventual occlusion of AVM after radiosurgery. The immunohistochemical characteristics of these spindle-shaped cells were identical to myofibroblasts, which are the activated forms of resting fibroblasts expressing contractile elements [5]. In a subsequent paper Schneider et al. presented similar results in AVM after radiosurgery [26].

The goal of the present study was to investigate histopathological alterations and compare them with treatment parameters, neurodiagnostic imaging and clinical follow-up data in vestibular schwannomas after LGK radiosurgery. Considering that the present series of 17 VS harboring patients who underwent microsurgical resection after LGK treatment expressed histopathologically more or less living tumor tissue remnants in the examined specimens, we have to accept that this patient population represents some kind of radiosurgical failure. Surviving VS islands were demonstrated in all cases mainly in the outer capsule or mantle zone, which is assumed to receive lower doses of irradiation at about the 50-60% isodose line [31]. If we take out from this group those 4 patients...
who were operated on in the latency period of the irradiation, the remaining 13 failures constitute less than 1% of the 1350 LGK treated cases in the Centre Gamma Knife, Hôpital La Timone since 1992. From the analysis of the available pathological material several pathophysiological mechanisms might be suspected behind the volume progression after radiosurgery. These processes include degenerative and proliferative changes in the tissue elements generated by the ionizing energy of focused gamma-beams. From a morphological point of view the biological effect of high dose irradiation on VS tumor tissues seems to evoke a double reaction. One object is the tumor parenchyma where the radiation triggers cell death either via coagulation necrosis or induced apoptosis. Immunohistochemical investigations (FVIII, CD31, CD34) revealed that the other target of radiosurgery is the stroma of the tumor, where it induces endothelial destruction and wall damage of the vessels with granulation tissue proliferation what organizes and clears up necrotic tumor tissue. Results of these immunohistochemical reactions demonstrate that the endothelial cell layer of the vessels is very sensitive and reacts early after high-dose irradiation of tumor tissue [22]. The previous observations supply human pathological data and support the experimental theory that microvascular endothelial cells are the primary targets of single high-dose irradiation [4, 23]. In the later stages it is suspected
to be replaced by hypocellular connective tissue reached in collagen bundles. The process seems to finish with hyaline degeneration and scar tissue formation that substitutes the destroyed parenchyma. Regarding that scar tissue prone to contraction it might promote shrinkage of VS after LGK treatment in the majority of cases. However in the failed radiosurgical tumors histological evidence were supplied in several instances for excessive hemorrhage and extensive necrosis. These might be consequences of vascular damages and could result in tumor volume enlargement presenting with radiological progression and clinical deterioration.

A notable finding of the immunohistochemical investigations was (FVIII, CD31, CD34) that the endothelial layer and the vessels’ wall were damaged during the irradiation latency period but the positivity recurred again beyond the latency period. This phenomenon suggests either that the reactivity of the endothelial cells (FVIII, CD31, CD34) and vascular smooth muscle elements (CD34) recover after the latency period, or there is de novo vessel genesis.

Another considerable observation was that proliferative capacity of the tumor cells (Ki67) was detected even beyond the latency period. This finding might be a consequence of a genetically highly active tumorous Schwann cell population what could resist to the generally used radiosurgical doses in the treatment of common VS.

Fig. 8. — a) Marked CD31 activity in the endothelial cells of tumor vessels in a non-irradiated control vestibular schwannoma (×100); b) Decreased CD31 reaction 6 months after radiosurgery (×100); c) No CD31 positivity could be detected 16 months following radiosurgery (×100); d) Returned CD31 reactivity 48 months after radiosurgery (×100).

Fig. 8. — a) Activité CD31 marquée des cellules endothéliales des vaisseaux tumoraux au sein d’un schwannome vestibulaire de contrôle non irradié (×100). b) Diminution de la réactivité CD31 6 mois après la radiochirurgie (×100). c) Absence de réactivité CD31 16 mois après la radiochirurgie (×100). d) Réactivité CD31 de nouveau 48 mois après la radiochirurgie (×100).
The analysis of this series revealed a patient presenting with a secondary glioblastoma multiforme in the temporal lobe on the same side 8 years after LGK treatment of his VS, therefore the issue of radiosurgery-induced brain tumors should be discussed. Recently a few reports have been published on brain tumors attributed to radiosurgery. In three patients, glioblastoma multiforme develop...
of central enhancement was observed by Prasad reflecting the biological response to radiosurgery. A loss in tumor volume and native to microsurgical resection for vestibular schwannomas [8]. Changes in tumor volume and decreasing continuously, unfortunately the theoretical possibility exists for potential long-term complications after radiosurgery. But as the number of treatments has been increased continuously, unfortunately the theoretical link between a treated target and a second tumor several cm away from the irradiated region. Therefore in our opinion it would be against the idea of radiosurgery to draw a direct causal link between a treated target and a second tumor several cm away from the irradiated region. But as the number of treatments has been increased continuously, unfortunately the theoretical possibility exists for potential long-term complications after radiosurgery.

Stereotactic radiosurgery is the principal alternative to microsurgical resection for vestibular schwannomas [8]. Changes in tumor volume and changes in the imaging properties of neoplasms reflect the biological response to radiosurgery. A loss of central enhancement was observed by Prasad et al. [24] in more than 50% of the treated cases. The earliest onset of this change in their material took place at 4 months post-treatment and it appeared as late as 4 years as well. Spiegelmann et al. have reported that early tumor enlargement after radiosurgery was always associated with a loss of central contrast enhancement on MR imaging [28]. It was thought to be the result of hyperacute tumor ischemia with edema and proved to be transient. Imaging studies demonstrated volume reductions in all these tumors later during the follow-up period. In a previous study we have found that the loss of central contrast enhancement on MR imaging appeared as early as 2.5 months after radiosurgery [31]. Histopathological investigations revealed coagulation necrosis in the tumor tissue behind the imaging changes, which might have been the consequence of vascular damage.

QUO VADIS?

Insomuch as pathophysiological mechanisms behind imaging changes and radiosurgical failure are still subjects of debate, further histopathological investigations and comparison with radiological findings would be desirable at every available case in the future. The role of functional examinations like positron emission tomography (PET) should be considered in the treatment planning and follow-up assessment of VS after radiosurgery. This sophisticated method detects sensitively metabolic changes of proliferating tissues like tumor or granulation tissue and was found to be useful in the dosimetry planning of radiosurgery by Levivier et al. [15]. The combination of morphological and functional imaging data with histopathological findings probably would promote better understanding the radiobiology of radiation effect on vestibular schwannomas after Gamma Knife radiosurgery. In this way it would serve more sophisticated treatment planning and could reduce the number of failed radiosurgery cases.

CONCLUSIONS

Results of the present histopathological study suggest that radiosurgery offends a double target during treatment of vestibular schwannomas. It seems to destroy directly tumor parenchyma either via coagulation necrosis or induced apoptosis in the neoplastic cells. Vascular channels of the connective tissue stroma are also involved in the radiobiological response evoked by the ionizing energy of gamma photons. Immunohistochemical investigations supply human pathological support to the experimental theory that vascular endothelial cells are the principal targets of single high-dose irradiation. These vascular changes could be relevant in VS regression together with necrotic or apoptotic tumor cell death after Gamma Knife treatment. The loss of central contrast enhancement of tumor tissue and MRI signal changes might be consequences of the vascular damage.

Proliferative capacity of tumor cells decreased after radiosurgery but still existed beyond the latency period. Endothelial destruction and vascular wall damage appeared early following radiosurgery but it was repaired after the latency period of irradiation.

Clinical pathological data do not support emergency craniotomy for patients with radiological progression but without clinical deterioration, mainly in the latency period. Further management of these patients should be based on consultations and common decisions by the radiosurgical and the microsurgical team.

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