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ARTICLE

PET Identifies Transitional Metabolic Change in the Spinal Cord Following a Subthreshold Dose of Irradiation*

Olga ÉSIK,[1] Miklós EMRI,[2] Szabolcs SZAKÁLL Jr,[2] Hans HERZOG,[3] Géza SÁFRÁNY,[4] Erzsébet LENGYEL,[5] András BOÉR,[6] Gabriella LISZKAY,[7] Lajos TRÓN,[2,8] Zsolt LENGYEL,[2] Imre REPA[9]

[1]Department of Oncotherapy, Pécs University of Sciences, Pécs, Hungary, [2]PET Center, University of Debrecen, Debrecen, Hungary, [3]PET Laboratory, Institute of Medicine, Research Center, Jülich, Germany, [4]National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary, [5]Department of Radiology, Section of Oncotherapy, Semmelweis University, Budapest, Hungary, [6]Department of Head and Neck Surgery, and [7]Department of Dermatology, National Institute of Oncology, Budapest, Hungary, [8]PET Study Group of the Hungarian Academy of Sciences, Debrecen, Hungary, [9]Department of Diagnostic Imaging and Oncoradiology, University of Kaposvár, Kaposvár, Hungary

Positron emission tomographic (PET) investigations were performed to obtain *in vivo* information on symptomless radiation-induced pathological changes in the human spinal cord. PET investigations were carried out prior to radiotherapy and during the regular follow-up in an early hypopharyngeal cancer patient (the spinal cord was irradiated with a biologically effective dose of 80 Gy_2), with [^{18}F]fluorodeoxyglucose (FDG), [^{11}C]methionine and [^{15}O]butanol as tracers; radiosensitivity and electroneuronographic (ENG) studies were also performed. A very low background FDG accumulation (mean standardized uptake values, i.e. SUV: 0.84) was observed in the spinal cord before the initiation of radiotherapy. An increased FDG uptake was measured 2 months after the completion of radiotherapy (mean SUV: 1.69), followed by a fall-off, as measured 7 months later (mean SUV: 1.21). By 44 months after completion of irradiation, the FDG accumulation in the irradiated segments of the spinal cord had decreased to a level very close to the initial value (mean SUV: 1.11). The simultaneous [^{15}O]butanol uptake results demonstrated a set of perfusion changes similar to those observed in connection with the FDG accumulation. The patient exhibited an extremely low [^{11}C]methionine uptake within the irradiated and the nonirradiated spinal cord during the clinical course. She has not had any neurological symptoms, and the results of central ENG measurements before radiotherapy and 2 months following its completion proved normal. Radiobiological investigations did not reveal unequivocal signs of an increased radiosensitivity. A transitory increased spinal cord FDG uptake following radiotherapy may be related to the posttherapeutic mild inflammatory and regenerative processes. The normal [^{11}C]methionine accumulation observed is strong evidence against intensive cell proliferation. The high degree of normalization of the temporarily increased FDG uptake of the irradiated spinal cord segments by 44 months is in good agreement with the results of monkey studies, which demonstrated a nearly complete recovery from radiation-induced spinal cord injury. (Pathology Oncology Research Vol 10, No 1, 42–46)

Keywords: radiotherapy; spinal cord; positron emission tomography; [^{18}F]fluorodeoxyglucose; [^{11}C]methionine; [^{15}O]butanol; radiobiology

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Correspondence: Olga ÉSIK, Department of Oncotherapy, Pécs University of Sciences, H-7624 Pécs, Édesanyák útja 17., Hungary. Tel: 36-72-536080, Fax: 36-72-536481,
E-mail: olga.esik@aok.pte.hu and www.esik-olga.hu

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Introduction

Very little information is available on symptomless radiation-induced pathological changes in the human spinal cord. Conventional staining of autopsy material from patients who had presented with transient Lhermitte's sign, but died from unrelated reasons, did not

reveal any pathological alterations in the cervical spinal cord.[1] However, moderate to mild demyelination and/or vascular injury were demonstrated following a single course of radiation treatment with 70.4-83.6 Gy, even in symptomless monkeys.[2-4] Similar findings were detected when an initial dose of 44 Gy or was followed 1–3 years later by a course of 39,6–66 Gy in monkeys.[4,5] An investigation of the postradiation spinal cord sequelae in these monkeys revealed that 61-100% regeneration can occur by 3 years after radiotherapy.[5]

As pathological examinations in human can be carried out only *post mortem*, noninvasive diagnostic methods are necessary to investigate mild, radiation-induced pathophysiological processes *in vivo*. MRI, a sensitive tool in the detection of demyelination or vascular changes, failed to demonstrate any pathological signs in Lhermitte's syndrome caused by different disorders,[6-8] accordingly, we were interested in finding another method suitable for the characterization of radiation-induced alterations. Positron emission tomography (PET) is a sensitive tool with which to establish the functional status of different organs, and we therefore tested the suitability of this method for the disclosure of mild radiation-related changes within the spinal cord.

This paper describes clinical investigations aimed at the characterization of radiation-induced changes in the spinal cord physiology through the use of [^{18}F]fluorodeoxyglucose (FDG), [^{11}C]methionine and [^{15}O]butanol PET; radiosensitivity and electroneuronographic (ENG) studies were also performed.

Patient and Methods

A 49-year-old woman was diagnosed to have an early hypopharyngeal cancer (T1 N2b M0) in 1998 allowing a successful partial hypopharyngeal resection and radical right cervical lymph node dissection. The surgical intervention was followed by external irradiation with 6 MV photons and 8 MeV electrons. The intention was to treat the tumor bed and the bilateral parajugular lymph nodes with a maximum midplane dose of 50 Gy, using daily right and left portals with an angled-down technique (2 Gy/fraction/day, 5 times a week). When a midplane dose of 40 Gy was reached, the field size was shrunk and the spinal cord was no longer exposed directly to the irradiation. The posterior part of the field was boosted by 10 Gy electrons. Thus, the radiotherapy involved a cervical spinal cord dose of 40 Gy, with a calculated biologically equivalent dose (BED) of 80 Gy_2, assuming /=2 Gy.[9]

The patient subsequently has not had any neurological symptom (no Lhermitte's sign either) and has been disease-free for 6 years. The results of central ENG measurements (bilateral neck somatosensory and magnetic evoked potentials) before radiotherapy and 2 months following its completion proved normal. Likewise, MRI investigations (1.5 Tesla, Magnetom Vision Plus or Magnetom Symphony, Siemens, Erlangen) did not reveal any spinal cord abnormality before radiotherapy or during the follow-up.

Before radiotherapy and during the clinical course, PET investigations were performed with a GE 4096 Plus scanner (General Electric, Uppsala, Sweden). The doses applied were 5.55 MBq/kg FDG, 9.25 MBq/kg [^{11}C]methionine and approx. 2 GBq/scan [^{15}O]butanol. Attenuation correction was based on transmission scans. Iterative reconstructions were made with a maximum likelihood algorithm[10] and the reconstructed image resolution was about 6 mm. For numerical comparisons, standardized uptake values (SUVs) were determined in the axial plane of the PET images by placing a region of interest (ROI) covering the cervical spinal cord between vertebral bodies C2 and C7. The average activity per milliliter in the ROI was found by applying a calibration factor obtained by scanning a uniform cylinder containing a known activity concentration. The uptake in this region was then corrected for the injected activity and the weight of the patient. The PET examinations had previously been approved by the Ethical Committee of the University of Debrecen and the patient had given her informed consent.

For image registration and fusion, we have used a stored follow-up CT scan of 3-mm axial slice thickness obtained with a Somatom Plus 4 tomograph (Siemens, Erlangen, Germany). Image registration was carried out by internal landmark matching technique, using only linear transformations with the help of the "Register" software (Montreal Neurological Institute).

The radiobiological investigations were approved by the Hungarian Health Care Scientific Council (6008/1/ETT/2002) and the patient again gave her informed consent. Two different experiments were performed to check whether an individual high radiosensitivity may have played a role in the development of radiation-induced spinal cord alterations in this patient. First, a primary fibroblast cell culture was established from a skin biopsy[11] and then, in a clonogenic assay,[12,13] fibroblasts were irradiated with different doses of radiation, and the survival rates were compared with the clonogenic survival of fibroblasts from 6 healthy subjects. Second, in a single cell electrophoresis (comet) assay,[14,15] whole blood was irradiated with 2 Gy of γ-radiation and comet analysis was performed either directly after irradiation to measure the initial DNA damage, or 4 h later to allow time for DNA repair and determination of the residual damage. Data evaluation was supported by the Komet Analysis System software package. The initial and the residual DNA damage in the lymphocytes of the patients were compared with the results obtained on 43 samples collected from healthy individuals.

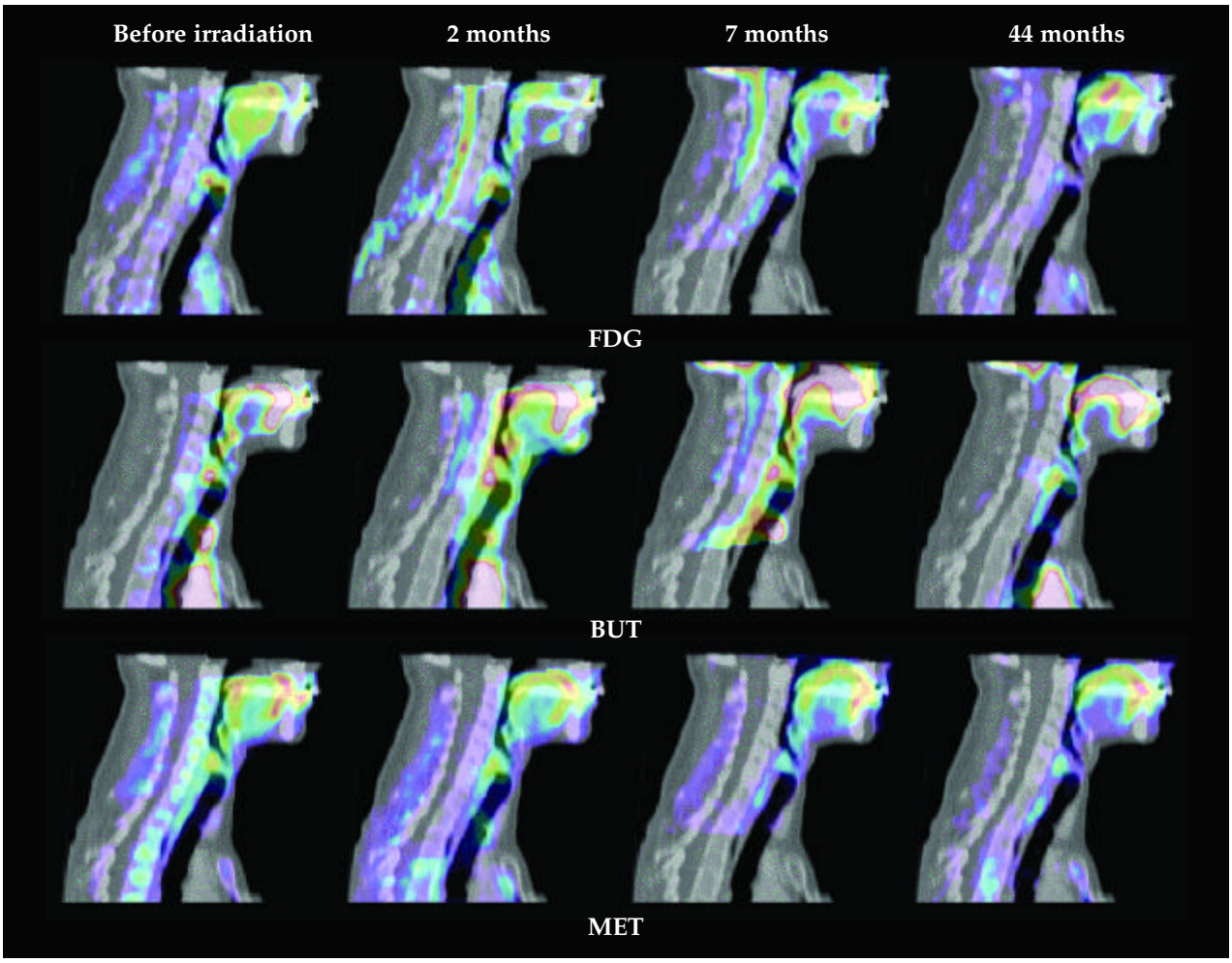

Figure 1. *Fused CT-PET images of the median sagittal section of the head and neck region using FDG (upper panel), [^{15}O]butanol (middle panel) and [^{11}C]methionine (lower panel), as tracers before irradiation and during the clinical course (2, 7, and 44 months following irradiation). The consecutive images display SUVs on an identical scale*

Results

A very low background FDG accumulation was observed in the spinal cord of this patient in 1998 (mean SUV: 0.84), before the initiation of radiotherapy (*Figure 1*, upper panel). An increased FDG uptake was measured 2 months after radiotherapy (mean SUV: 1.69), which was followed by a decline, as measured 7 months later (mean SUV: 1.21). The FDG accumulation in the irradiated segments of the spinal cord had decreased to a level very close to the initial value by 44 months after completion of irradiation (mean SUV: 1.11).

The simultaneous [^{15}O]butanol uptake results demonstrated a set of perfusion changes similar to those observed in the FDG accumulation (*Figure 1*, middle panel). The patient had an extremely low [^{11}C]methionine uptake (*Figure 1*, lower panel) within the irradiated and the nonirradiated spinal cord which did not change during the clinical course (the mean SUVs were between 0.25 and 0.35). The bone marrow uptake disappeared from the irradiated vertebral bodies C 2-Th 3 (C 1 has practically no body).

In the fourth year of the clinical course, the fibroblasts of the patient displayed a slightly increased radiation sensitivity relative to those of the healthy controls (*Figure 2*). In the comet assay, no essential difference was found between the DNA repair capacity of the patient's lymphocytes and those of the controls.

Discussion

The few studies that have been published on PET of the spinal cord revealed a very low physiological FDG uptake, due to the considerable proportion of white matter with low metabolic activity relative to the small bulk of the gray matter.[16-20] The spinal cord usually also exhibits a rather low [^{11}C]methionine uptake, in consequence of the slow cell

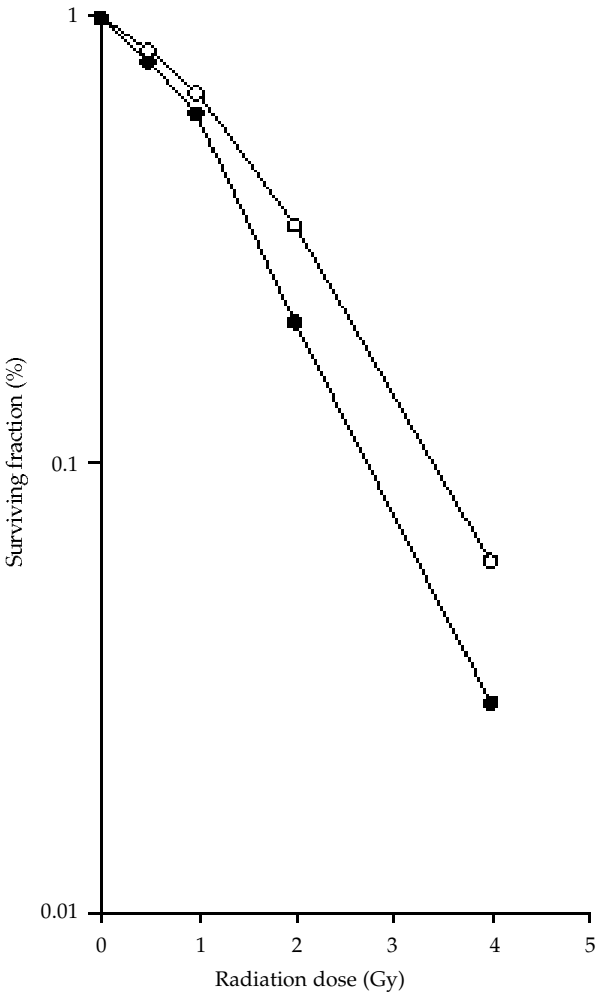

Figure 2. *Survival curves for primary fibroblast cells. Filled circles: fibroblasts from the present patient; open circles: average survival of fibroblasts from 6 healthy children*

turnover.[19,21-24] A close direct relationship was described between the regional blood flow and the glucose metabolism of the spinal cord,[23,24] which was very similar to the coupling of these parameters observed in brain studies.[25]

After radiotherapy of the spinal cord with a BED of 80 Gy_2, MRI and conduction velocity investigations of the present patient did not disclose any pathological sign of abnormalities. At the same time, the irradiated segment of the spinal cord exhibited increased FDG and [^{15}O]butanol uptakes, which continually fell off during the 4-year clinical course. The parallelity in the changes of the accumulation of these two tracers is in complete agreement with previous findings.[23,24] The spinal cord exhibited a permanently low methionine uptake, equal to the physiological level.

Wilmshurst et al. observed an increased spinal cord FDG uptake following radiotherapy, without any neurological sign.[19] Their patient was irradiated for a recurrent, previously resected low grade cauda equina ependymoma (benign papilloma; the radiation dosage was not stated). Two months after irradiation, FDG PET investigation revealed an increased tracer uptake (SUV 5.2); no tumorous sign was detected by MRI during the subsequent 3-year clinical course. The difference in absolute postirradiation values between our findings and those of Wilmshurst et al. may be explained by several facts. The doses applied may have been dissimilar, as the spinal cord of our patient received only 40 Gy, and the cauda equina tumor probably required a higher radiation dose. Although the radiobiological investigations of our patient did not reveal unequivocal signs of an increased radiosensitivity, a possible difference in the individual responses to radiation cannot be ruled out. Finally, the exact time protocol of the FDG imaging was not the same in the two cases either. It is noteworthy that an increased FDG uptake in the brain was reported earlier following intensive radiotherapy of malignant brain tumors.[26]

The concordant results allow speculation concerning the pathomechanism of the transiently increased FDG accumulation within the irradiated spinal cord. An elevated glucose metabolic rate can be a sign concomitant with cell division or inflammatory processes stemming from the pathological responses of the central nervous system (CNS) to ionizing radiation.[27-33] Demyelination (mainly related to the loss of oligodendrocytes and glial progenitor cells), one of the induced pathological processes, inevitably results in consecutive gliosis and astrocytosis (Type 1 lesion), both requiring extra energy consumption. It is generally accepted that energy-demanding remyelination is a clear prerequisite for functional regeneration.[34] Jeffery reported that normalization of a toxin-induced locomotor deficit of the spinal cord accompanied by spontaneous remyelination.[35] Recent studies have revealed that many regions of the adult CNS contain neuronal progenitors that have the ability to generate new neurons and glia.[36,37] Sasaki et al. described that transplantation of isolated bone marrow fraction led to the repair of X-ray-demyelinated adult rat spinal cord axons, apparently as a result of the bone marrow stem cells undergoing differentiation into myelin-forming cells.[38] It has also been shown that the number of oligodendrocyte progenitor cells increases following mechanical spinal cord injury.[39]

Vascular (Type 2) lesions include endothelial cell damage, preferentially in capillaries and venulae, which is usually accompanied by a chronic inflammatory reaction involving mononuclears (lymphocytes, mainly T cells, macrophages and microglia), and often by fibrinoid necrosis of the vascular wall (radiation vasculitis), resulting in an increased energy demand. Eventually, thickening of the vessel walls, telangiectasia and thrombosis/occlusion evolve. Besides white matter reactions, gray matter sequelae (neuronal degeneration, chromatolysis, and a coarse tigroid appearance of the Nissl substance) may also occur in the anterior and posterior horns of the myelon,[28-31,40] but

the gray matter *per se* is less radiosensitive than the white matter,[28-30] and it is not clear whether this may contribute or not to the elevated glucose metabolic rate.

Thus, the temporarily increased FDG uptake after radiotherapy may be related to subclinical, transitory demyelination and vascular inflammation, triggered by a not too high dose of irradiation. The background level of [^{11}C]methionine accumulation in the spinal cord segment exposed to radiation can be regarded as strong evidence against intensive cell proliferation. The almost complete disappearance of the temporary increase in the FDG uptake of the irradiated spinal cord region by the 44th month is in accordance with the finding that the monkey spinal cord virtually completely recovers from radiation damage in 3 years.[5]

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