CHAPTER 1

Introduction and scope of the thesis
CHAPTER 1

Introduction

Fifty percent of all cancer patients are treated with radiotherapy, as a single treatment or in combination with surgery or chemotherapy. The main goal of cancer treatment is to cure the patient by eradicating the tumor with minimal morbidity to critical organs and healthy tissues. The tolerance for radiation of the surrounding healthy tissue dictates therefore the total prescribed dose to the tumor. One of the most critical organs in radiation therapy is the spinal cord. Depending on the site, dose and the irradiated volume one can observe damage of the spinal cord resulting in sensory and/or motor deficits. Minor radiation-induced damage is often subclinical and reversible. Transient demyelination after irradiation of the cervical spinal cord is clinically manifest after 2-37 weeks and is characterized by electric ‘shock like’ sensations into the arms, the spine and the legs after flexion of the neck (Lhermitte’s sign). In most cases these symptoms resolve within 6 months (1) but rarely Lhermitte’s sign precedes permanent radiation myelopathy (2). Objective signs and symptoms of radiation myelopathy include changes in gate, spasticity, weakness, hemiparesis, Brown-Sequard syndrome, hyperreflexia and Babinski signs on neurological exam. No combination of signs or symptoms distinguish radiation myelopathy from myelopathies of many other etiologies (2). The most devastating radiation-induced damage to the central nervous system (CNS) is white matter necrosis (WMN). In a recent review article (3), it is quoted that radiation-induced necrosis in the brain and spinal cord were reported by Fisher and Holfelder in 1930 and Ahlbom in 1941. This type of damage occurs after months to years post treatment with permanent and severe neurological deficits such as gait disturbance, hemiplegia and paralysis. Latencies of less than 6 months are rare and patients who received a dose less than 50 Gy in 2 Gy daily fractions are generally not at risk. Since the WMN is irreversible, the radiation-oncologist has to be very careful in treating tumors that are in close proximity to the spinal cord and limits therefore tumor dose escalation. Only limited clinical data about the incidence of radiation myelopathy are available. The clinically applied spinal cord tolerance dose is not
Introduction and Scope of the Thesis

based on well established clinical data. To overcome the lack of data, many animal studies have been carried out to get more knowledge about dose-volume effects and mechanisms involved in radiation-induced damage to the spinal cord. The anatomical similarities between the human and rat spinal cords make the rat spinal cord a suitable model to study dose-volume effects.

The spinal cord

Macroscopic anatomy

The spinal cord is the elongated, approximately cylindrical part of the CNS which occupies most of the diameter of the cranial two-thirds of the vertebral canal. In humans it extends from the level of the cranial border of the first cervical vertebra to the caudal border of the second lumbar vertebra. The average length in a Caucasian male is about 45 cm., in the female from 42 to 43 cm while its weight about 30-38 g (4,5). The diameter varies from 10 mm at the thoracic level to 12-14 mm at the cervical and lumbar levels.

The cord is ensheathed by three meninges which are separated by the subdural and subarachnoid spaces. These spaces are filled with cerebrospinal fluid. On a transverse section, the gray matter and white matter are clearly distinguishable. The butterfly shaped gray matter consists of a complex intermingling of nerve cells and their processes with neuroglia and blood vessels. The white matter consists of nerve fibers, neuroglia, blood vessels and a small population of glial stem cells. The white color is due to the predominance of myelinated nerve fibers. The majority of the fibers are longitudinally orientated in contrast to the fibers in the gray matter which are more or less transversely orientated. The descending corticospinal tracts or pyramidal tracts are responsible for facilitating locomotion. In the human spinal cord, the axons descend from the cortex and at the junction between the medulla and spinal cord about 80% of the fibers cross to the contralateral side in the pyramidal decussation. As a result there is a lateral corticospinal tract (crossed) and an anterior corticospinal tract (uncrossed). On transverse section the lateral corticospinal tract occupies an oval
area anterolateral to the posterior gray column whereas the anterior corticospinal tract lies alongside the anterior median fissure (Fig. 1).

Figure 1. A schematic transversal section of the human cervical spinal cord (a) and the rat cervical spinal cord (b) with the locations of the corticospinal tracts.

In the rat spinal cord the location of the corticospinal tract is slightly different from the human spinal cord. Most of the corticospinal tract fibers cross the midline in the pyramidal decussation. This crossed corticospinal tract is located in the ventral portion of the dorsal funiculus (6). A small number of uncrossed fibers
is located in the ipsilateral ventral side of the cord (7). All other ascending and descending tracts are left out of this overview for simplicity.

The blood supply of the spinal cord consists of three major arteries. The anterior spinal artery (ASA) is located in the anterior median fissure and the two posterior spinal arteries (PSA) are located just posterior to the dorsal root entry. The central branches of the ASA supply two-thirds of the cross-sectional area of the spinal cord. The remaining parts of the spinal cord are supplied by numerous small radially directed vessels derived from the posterior spinal arteries and from the vessels forming the plexus in the pia mater (4).

In the rat spinal cord, Koyanagi et al. (8) showed that the sulcal arteries supply most of the gray matter (GM) and white matter (WM) in the ventral and lateral spinal cord. The posterior GM and WM are fed from the posterior spinal arteries (8). The investigators observed in the GM a butterfly-shaped rich capillary network, whereas the anterior and lateral white columns were characterized by radially oriented vessels. The posterior column contains two large veins for drainage of the posterior columns, medial posterior GM and posterior GM. Other parts of the cord are drained by the sulcal veins and radial veins (8). The arterial supply results in a centripetal and centrifugal blood flow (BF) pattern in the spinal cord. In the white matter and in the dorsal half of the gray matter the local BF is mainly provided by the centripetal system whereas the ventral part of the gray matter derives its flow from the centrifugal system. Regionally, the vascular density is higher in the GM than in the WM and in the ventral half of the GM than in the dorsal half (9,10). The ratio of vascular density and blood flow in GM to WM is about 3:1 (9,10,11). The BF in the WM has a relatively homogeneous pattern whereas the GM has a more variable blood flow with topography (9,10).

The CNS is protected from the blood supply by the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB). This barrier is a physical and metabolic barrier between the CNS and the systemic circulation. It serves to regulate and protect the microenvironment of the brain and spinal cord (12). The morphologic basis of the BSCB resides in the presence of tight junctions between endothelial cells, the lack of fenestrations and the paucity of transcellular transport via
membranic bound vesicles (12,13). The microvascularity in the CNS has intimate contact with astrocytes. The astrocytic end feet form a complete layer around the basal lamina of the endothelial cells (12,13).

**Microscopic anatomy**

The nerve cell is responsible for the signal conduction. It consists of a cell body, a long axon and short dendrites. The nerve fibers can be ensheathed by myelin. The myelin sheath around an axon is protecting the nerve fibers and facilitates a quick propagation of the nerve impulses. The diameter of a myelinated axon ranges from 1 to 20 µm; unmyelinated nerve fibers have a diameter of approximately 1 µm (5).

In the GM as well as in the WM the nerve cells and fibers are surrounded by and embedded in the neuroglial cell population. Three different neuroglial cell types can be identified: oligodendrocyte, astrocyte and microglial cell. In the past these cells were thought to be the supportive tissue (or glue) without specific functions. However, in the last decades more knowledge has been gathered about these cells and their specific functions in the CNS.

Most oligodendrocytes are located in the white matter where their primary role is to enwrap the internodal segments of axons to form myelin sheaths that facilitates fast conduction of nerve impulses (14,15). One oligodendrocyte is capable of myelinating up to 60 different axons (15). Damage to a single oligodendrocyte may result in dysfunction of many different axons.

The astrocyte is the most prevalent cell type in the CNS comprising more than half the brain volume and outnumbering neurons by approximately 9:1 (16). The astrocyte has different functions in the healthy CNS such as regulating the composition of the extracellular environment, clearing an excess of neurotransmitters, regulating the extracellular ion concentration, modulating the formation and efficiency of synaptic connections (15,17,18) and are capable of secreting a wide variety of cytokines, proteases and other bioactive molecules that affect the vasculature, neurons and the oligodendrocyte lineage (16,18). The astrocyte contributes both the structural and functional integrity of the blood-
Introduction and Scope of the Thesis

brain barrier (17) and it plays a critical role in the reaction and response of CNS to radiation induced injury (16).

The microglial cells comprise 10% of the cells in the brain parenchyma (19) and are both developmentally and functionally related to cells of peripheral monocyte-macrophage lineage (20). Microglia have the potential to become phagocytes (21). As a cell of macrophage potential it needs appropriate stimulation to enter a stepwise transformation for developing features and functions of a full-blown macrophage (22). Microglia are involved in local inflammatory responses, are capable of proliferation, phagocytosis and secretion of hydrolitic enzymes, lipid metabolites and oxygen radicals (16). A resent study by Yokoyama suggests a novel role of microglia as multipotential stem cells to give rise to neurons, astrocytes or oligodendrocytes (19). After irradiation of the brain increases in the number of microglial cells have been suggested to be associated with tissue damage (16). In the rat cervical spinal cord the microglial population in the gray matter showed a rapid and marked reduction after a single dose of 25 Gy that persisted for at least 6 weeks (23).

The definition of CNS stem cells is under debate: most researchers are guided by properties of stem cells in other systems such as multipotency, high proliferative potential and self-renewal (24) and it is difficult to define precisely the CNS class. The multipotent stem cells can generate neurons and glial cells whereas the adult oligodendrocyte progenitor cell is restricted to generating glial cells (24). The lack of a specific marker makes the identification of CNS stem cells a problem (25). What can be agreed on is that multiple stem cells exist in the brain that is based on the interpretation of different data using diverse techniques and not on a unique molecular marker of CNS stem cells (25). The first specialized oligodendrocyte progenitors in the spinal cord are generated at a discrete locus in the ventricular zone in the ventral half of the cord and subsequently proliferate and migrate throughout the spinal cord before differentiating into oligodendrocytes (26).
Animal studies of dose-volume effects in spinal cord

Generally, it is assumed that the tolerance dose (a dose above which a specific complication occurs) for many normal tissues increases by decreasing the irradiated volume of that tissue. It is important to discriminate between structural tissue tolerance and functional tolerance (27). Structural tissue tolerance depends on the cellular radiation sensitivity and the ability of clonogenic cells within a defined volume to maintain the mature cell population above a critical level. Functional tolerance depends on whether the organ as a whole can contribute to its function. This is determined by tissue organization as well as cellular sensitivity. The behavior of the tolerance dose as a function of irradiated volume is strongly organ dependent (28,29). A dose-volume effect refers to a situation in which a graded response shows a volume effect if the change in response is not proportional to the change in irradiated volume (30). When studying dose-volume effects it is of utmost importance to determine a well defined response. The response in most studies on dose-volume effects in the spinal cord is radiation-induced paralysis. In the rat model, this response is always due to white matter necrosis when it occurs within 210 days after irradiation (31).

Since much of the knowledge for clinical dose-volume effects in the spinal cord is still based on dogma (32), a number of animal studies in rodents, dogs, pigs and monkeys have been undertaken to investigate dose-volume effects in the spinal cord.

In adult rats, irradiation of 1 cm up to the full length of cervical and thoracic cord (6 cm) did not show significant differences in tolerance doses. The ED50 values ranged from 21 Gy to 22 Gy (32). However, a reduction in irradiated cord length demonstrated a steep rise in isoeffective doses. Hopewell et al. (33) observed after single dose irradiation of the rat cervical spinal cord a distinct relationship for dose-related paralysis developing within 210 days and field size. The ED50 increased from 21.5 Gy to 30 Gy to 51 Gy for field sizes of 16, 8 and 4 mm. For animals developing paralysis beyond 210 days of follow-up, this relationship was less clearly defined. The ED50 increased from 20.0 Gy to 25.6 Gy
for a 16 mm field and a 4 mm field, respectively. Histological investigations showed that the radiation-induced paralysis which developed within 210 days was due to white matter necrosis whereas paralysis beyond 210 days was based on vascular damage.

In a study with pigs, Van den Aardweg et al. (34) observed a small dose-volume effect for paralysis due to white matter necrosis after irradiating 2.5, 5.0 and 10.0 cm of cervical spinal cord. At ED\textsubscript{50} levels, there was only an increase of 1.3 Gy from 27.0 to 28.3 Gy when the length was reduced from 10 to 2.5 cm. This small difference was no longer significant at probability levels below ED\textsubscript{10}.

Schultheiss et al. (35) treated rhesus monkeys with clinically relevant field sizes and fractionation schedules using 2.2 Gy per fraction. Three groups were irradiated with 8 cm fields to total doses of 70.4, 77 and 83.6 Gy. Two additional groups were irradiated to 70.4 Gy using 4 and 16 cm fields. The incidence of myelopathy after a total dose of 70.4 Gy increased from 15% to 20% to 37.5% for field sizes of 4, 8 and 16 cm, respectively (35). The radiation doses inducing 50% (ED\textsubscript{50}) and 1% (ED\textsubscript{1}) incidence of myelopathy were 76.1 ± 1.9 Gy and 59.1 ± 5.5 Gy (36).

The only study that showed a significant dose-volume effect was performed in dog spinal cord by Powers et al. (37). The investigators irradiated thoracic spinal cord segments of 4 and 20 cm using a fractionated schedule of 4 Gy per fraction. The dose-response curves for paralysis showed a large shift to higher doses from 54 Gy for the large field to 78 Gy for the small field. However, the shift of the dose-response curves for the occurrence of severe pathological lesions was less pronounced. The dose required for a 50% response (ED\textsubscript{50}) for pathological lesions was 8.3-15.0 Gy higher when a 4 cm length was irradiated than was the case when a 20 cm length was irradiated. The observed large volume effect might be due to inaccurate positioning procedure. An inaccurate positioning procedure in fractionated irradiation experiments has more impact on the effective total dose for the 4 cm than for the 20 cm volume. Assuming a Gaussian probability distribution for the position of the animal the average total dose distribution can be calculated for both field sizes. In a modeling exercise we found that an assumed
positioning inaccuracy of 1.5 cm could explain the published ED\textsubscript{50} values for severe lesions.

In an attempt to describe the influence of the irradiated volume on the tolerance dose, several mathematical models have been generated. Some examples are the critical-volume model that assumes the presence of a certain functional reserve in the organ and the critical-element model that assumes a full serial organization of the organ whereas the seriality model of Källman assumes that the organ is a mixture of both parallel and serial linked chains. A comparison of these various models and a critical analysis of their validity has recently been published (38,39). Existing models are useful describe the data from spinal cord studies with uniform dose-distributions for various lengths, but are inadequate for non-uniform dose distributions.

**White matter necrosis**

In the literature, opinions about the pathogenesis of white matter necrosis have often focused on either a primary glial or a primary vascular origin, or a combination of both (31,40,41,42,43). White matter necrosis is characterized by demyelination, loss of axons, focal necrosis, and liquefactive necrosis, after single doses of \( \geq 20 \text{ Gy} \) (31). At the low end of the dose range, the lesions may remain as scattered foci. After higher doses the individual foci rapidly expand and coalesce into larger necrotic areas. Hopewell et al. (33) showed that the extent of white matter necrosis is less in a large volume (16 mm) than in small volumes (8 and 4 mm) at isoeffective doses for neurologic damage. In the 16 mm volume experiment, white matter necrosis was randomly distributed and no extensive lesions were seen in dorsal, ventral, and lateral white matter columns. In recent years it has become clear that endothelial cells and glial cells are not the only targets and components in the process that eventually results in WMN. During the latent period that precedes the neurological signs there is an active phase where vasoactive substances, cytokines (CK) and growth factors (GF) play important
roles in inter- and intracellular communication (44). Little is known about the orchestration and the time-course of the involved processes preceding WMN.

**Effects on endothelial cells and glial cells**

Three hours after a single dose of 15 Gy, the synthesis of prostaglandin-E$_2$, thromboxane and prostacyclin is increased and lasts for at least 3 days (45). After 7 and 14 days the synthesis of these substances decreased significantly. This was followed by an increased thromboxane production and a simultaneous reduction in prostacyclin production at 28-240 days. After more than 120 days, the serotonin production was increased. Two phases of increased vascular permeability were associated with these changes at 24 hours and 120-240 days postirradiation (45). Programmed cell death or apoptosis is observed in the mouse spinal cord after 4 hours (46) and in the rat spinal cord after 8 hours, and returns to control levels at 24 hours (46,47,48,49). Predominantly the oligodendroglial and endothelial cells show apoptosis and there is no evidence that astrocytes undergo apoptosis (47,48). The reduction of endothelial cells by apoptosis is dose-dependent with an associated dose-dependent disruption in blood-spinal cord barrier (49,50). The disruption of the blood-spinal cord barrier (BSCB) results in a transiently increased vascular permeability (49,51). After 7 to 14 days the endothelial cell density increases with a recovery of the BSCB function at 1 week after irradiation (50). The acute BSCB disruption after irradiation is mediated by the acid sphingomyelinase (ASMase) but not by the p53 pathway (50) in contrast to oligodendroglial apoptosis which is p53 dependent (52). The influence of early apoptosis on the development of white matter necrosis is yet unclear. Paris et al. (53) observed that radiation damage to gastrointestinal (GI) stem cell clonogens is a consequence of extensive microvascular injury. The GI syndrome which results in organ failure and death was prevented when endothelial apoptosis was inhibited pharmacologically by intravenous basic fibroblast growth factor (bFGF). The endothelial, but not crypt, cells expressed FGF receptor transcripts, suggesting that the endothelial lesion occurs before crypt stem cell damage in the evolution of the GI syndrome.
Direct evidence for vascular changes occurring before necrosis has been shown following irradiation of the brain (54,55) and spinal cord of the rat (11). The vascular changes consisted of endothelial cell nucleus enlargement, blood vessel dilatation, blood vessel wall thickening, and astrocyte hypertrophy. Studies in the rat spinal cord with 10Boron capture agents showed evidence for an apparent lack of involvement of glial progenitor cells in the pathogenesis of white matter necrosis. These experiments allowed preferential irradiation of blood vessels and relative sparing of the parenchyma and showed white matter necrosis comparable with that after irradiation with thermal neutrons alone (56).

Oligodendrocytes undergo also dose-dependent apoptosis within 24 hours after irradiation (47,48,57) followed by a decline of the oligodendrocyte cell density (57,58). After 22 Gy, the decline of the oligodendrocyte density is not restored to normal levels and the process of demyelination is reflected in a significant decrease of the proteolipid protein (PLP) gene expression after 4 weeks followed by some recovery and again a decline 2-3 weeks before paralysis (57). At a lower dose of 8 Gy, the demyelination is less pronounced. The role of oligodendrocyte progenitor cells (OPC) in the pathogenesis of white matter necrosis is not clear. It has been shown that the distribution of OPC’s is non-uniform in the spinal cord. In the adult rat spinal cord OPC’s are located in the outer circumference of the cord with no significant differences between dorsal, lateral or ventral regions (59). Several studies showed that areas depleted of progenitor cells by irradiation repopulated slowly by OPC’s from adjacent unirradiated areas (60,61,62). Hink et al. showed that depletion of OPC’s in a rat spinal cord segment occurred after a single dose of 40 Gy (62). The migration distance of OPC’s is limited to approximately 2 mm (61).

**Cytokines and growth factors**

During the latent period there is a complex intra- and intercellular communication where released cytokines (CK), growth factors (GF), vasoactive substances play an important role(44,63,64,65). Advances in cell biology have shown that CK and GF are involved in the pathogenesis of radiation induced injury.
of the central nervous system (16,44,66,67,68). Astrocytes and microglia are the mean sources of CK and GF that regulate modulate oligodendroglial proliferation, differentiation, migration, survival and the function of neurons (16,67). Insulin-like growth factor-1 (IGF-1) is an important survival factor for O-2A progenitor cells and seems to regulate the permeability of the blood-brain barrier whereas Platelet-derived growth factor (PDGF) stimulates survival of progenitors only. Vascular endothelial growth factor (VEGF) has besides the predominant role of growth factor in angiogenesis also direct effects on neurons and glial cells by stimulating growth, survival and axonal outgrowth (69). After irradiation of the rat cervical spinal cord, there was a dose-dependent hypoxia and VEGF up-regulation in reactive astrocytes (70). This VEGF up-regulation is associated with further increase in vascular permeability and BSCB disruption. Paris et al. (53) observed that radiation-induced gastrointestinal (GI) syndrome was prevented by administering basic fibroblast growth factor (bFGF). Similar modulating effects with growth factors were observed after irradiation of the mouse spinal cord (46) and rat spinal cord (44,68). In mice, the intravenous injection of bFGF showed a significant reduction of radiation-induced apoptosis (46). Platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) reduced the radiation myelopathy (RM) rates after irradiation of the rat spinal cord. Combining the IGF-1 with bFGF showed a further decrease of the incidence of RM (44,68).

After irradiation of mouse brain, proinflammatory cytokines like tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) are released by astrocytes and microglia (67,71,72). Only 2 hours after 25 Gy single dose irradiation of the mouse midbrain, an increase of mRNA levels of TNF-α and IL-1 was observed and decreased after 24 hours (67). TNF-α prevents differentiation of oligodendrocyte progenitor cells towards oligodendrocytes in vitro and it might cause damage to endothelial cells resulting in an increased vascular permeability and inhibition of cell proliferation (67). There are also data that suggest the involvement of apoptosis-mediating enzymes caspase-1 and caspase-3 only 3 hours after in vitro irradiation of oligodendrocytes.
chapter 1

**Latent period**

The latent period for white matter necrosis is the time between the irradiation and the appearance of paralysis and is dependent on intrinsic (strain, age and blood-pressure) and extrinsic (LET and oxygen) variables. Intra-strain variations of latent periods for necrosis-related endpoints are usually limited to a few weeks (43). Age of the animals of the same strain will influence the latent period as is observed in rats. In adult rats, doses above 20 Gy will induce paralysis due to white matter necrosis within 7 months (31). After doses below 20 Gy, mainly vascular lesions are induced and these vary greatly in extent and time of onset. The latent periods vary from 7 months to more than 1.5 years (31).

The age at the time of irradiation has a clear effect on the latent period (73) and increases almost linear at ages up to 8 weeks. The latent period after irradiation is generally assumed to be determined by the turnover time of the functional cells and the critical cell number below which the tissue fails to maintain functional integrity (73). In experiments with juvenile rats it was shown that the latent periods increased almost linearly from about 2 weeks after irradiation at the age of 1 week to about 200 days after irradiation at the age of 8 weeks (73). Similar results have been reported by Geyer et al. (74). This increasing latent period with age in juvenile rats is probably a result of increasing lifetime of the functional cells up to the age of 7–8 weeks. In adult rats, a general observation is a decreasing latency with increasing dose (after single doses from 25 to 60 Gy) of about 2 days per Gy (43).

**The influence of radiobiological concepts on radiation-induced CNS toxicity**

**Total dose and fraction size**

The tolerance or iso-effective total dose for white matter necrosis of the spinal cord is affected by the number of fractions in which the total dose is applied. The iso-effective total dose increases when the dose per fraction decreases. This relationship between the iso-effective dose and dose per fraction can be described
by the linear-quadratic model (LQ-model: Biologically Effective Dose = $D[1 + d/(\alpha/\beta)]$ where $D$ is the total dose in $n$ fractions of size $d$). The relationship between the iso-effective total dose and fractionation is steeper for late-responding tissues such as the spinal cord than for acutely-responding tissues such as the skin. The $\alpha/\beta$ ratio in the LQ-formula is a parameter of the fractionation sensitivity of a specific tissue. The $\alpha/\beta$ ratio is low for late-responding tissues and is approximately 2 Gy for spinal cord. This low value indicates a relatively large fractionation sensitivity suggesting that hyperfractionation would reduce the risk of myelopathy if high radiation doses are to be delivered to the spinal cord (36).

**Time factor**

Unexpected irreversible neurological deficits were observed in a clinical study on continuous hyperfractionated accelerated radiotherapy (CHART). Patients received 3 fractions of 1.5 Gy per day (6 hour interval) for a continuous period of 12 days. The schedule was predicted to be safe based on a low $\alpha/\beta$ ratio for late effects and a repair half time of 1-1.5 hours as found earlier in rodents (75). However, four out of 74 patients developed radiation myelopathy (76).

Later experimental studies on repair kinetics after external beam irradiation of the adult rat spinal cord showed that the repair of sublethal damage has a biphasic pattern with a fast and slow repair component of 0.7 hour and 3.8 hours, respectively (77). These data showed that delivering 2 fractions per day at 6 or 8 hour intervals instead of one fraction a day led to an approximately 16% and 13% reduction in the tolerance. After continuous interstitial irradiation, Pop et al. (78) observed a biphasic pattern of repair kinetics as well with a fast component of 0.15 hour and a slow component of 2.44 hours. The unexpected high incidence of radiation myelopathy in patients treated with CHART can at least partly be attributed to compounding incomplete repair between fractions (36). In general, it is advised to use interfraction time intervals of at least 6-8 hours (36,78), and preferably only include the cord only once per day in an accelerated treatment schedule.
**Reirradiation**

Until recently it was assumed that retreatment of the spinal cord after a curative course of irradiation would result in a high incidence of myelopathy. In contrast to this clinical dogma, experimental data from rat studies showed that the spinal cord has a large capacity to recover from occult radiation injury \((79,80,81,82)\). In a definitive study on rhesus monkeys, Ang et al. \((83)\) retreated the cervical and upper thoracic spinal cord after an initial course of 44 Gy in 2.2 Gy fractions. The monkeys received a second radiation course after 1, 2 or 3 years. The second course was either 57.2 Gy in 26 fractions or 66 Gy in 30 fractions with cumulative doses of 101.2 Gy and 110 Gy, respectively. The recovery estimates at the ED\(_5\) level were 27.1-33.6 Gy (62-76%), 32.3-37.6 Gy (73-85%) and 40-44.6 Gy (91-101%) of the initial dose after 1, 2 and 3 years. In a literature review on clinical data, Nieder et al. \((84)\) indicated that cumulative doses of 65-68 Gy in 2 Gy fractions are safe when the first course does not exceed 45 Gy. These are very conservative estimates and further clinical studies are needed to establish the tolerance levels for retreatment conditions.

**Scope of the thesis**

In radiotherapy the dose to the tumor is often limited by the tolerance dose of the surrounding normal tissue, especially when the tumor is located near a critical organ like the spinal cord. New conformal techniques like IMRT, proton therapy are used to minimize the irradiated volume of normal tissue. However, the introduction of these new radiation techniques brings new questions to solve. IMRT with photons is worldwide available now. Generally, IMRT involves more fields than conventional radiotherapy with photon beams and as a consequence a larger non-target volume is irradiated with lower doses. In addition, the number of monitor units per Gy target dose is significantly higher and therefore the total-body exposure (integral dose) due to leakage radiation is increased. Further, the dose outside the planning target volume and applied to the normal tissues and critical organs is more inhomogeneously distributed than with conventional
radiotherapy. One way to reduce the risk is the use of proton beams instead of photon beams. Protons have physical properties that make it possible to minimize the non-target volume that is exposed to low and intermediate doses. Another advantage of proton beams is the substantially smaller integral dose. Despite these advantages of protons, the clinical implementation is held up because the costs are much higher than for a photon beam facility. To convince national health services of the benefits of proton beam irradiation, more knowledge about treatment optimalisation has to be gathered. One way to quantify the possible advantage of a certain technique is to obtain data on the dose-volume effects for different organs.

In this thesis we studied the dose-volume effects in the spinal cord and investigated what effects inhomogeneous dose distributions have on the tolerance dose of spinal cord. The rat cervical spinal cord was used to study these effects. Since proton beams may deliver sharp demarcations with good spatial accuracy, these particles are very suited to study dose-volume effects in small animals with high precision. We started the investigations by homogeneous irradiation of four different spinal cord lengths and estimated the dose-response curves for paralysis due to white matter necrosis. Then we substituted the homogeneous dose distributions for inhomogeneously arranged dose distributions. To determine a possible effect of the applied dose distributions, the data were compared with the data from the homogeneously irradiated animals.

It is known from literature and previous photon experiments that radiation-induced white matter necrosis has a preferential location in specific parts of the white matter. These observations were the basis for the investigation of a variable dose distribution over the transversal cross section of the spinal cord using paralysis and subsequent white matter necrosis as determined by histology as endpoints. Subsequently, the lateral and middle parts of the white matter were selectively irradiated and the tolerance doses were estimated.

The results of all experiments in combination with data from the literature are the basis of a hypothesis on the mechanisms involved in the radiation-induced damage and pathogenesis of white matter necrosis in the rat cervical spinal cord.
References

Introduction and Scope of the Thesis


64. Nieder C, Price RE, Rivera RE et al. Both early and delayed treatment with growth factors can modulate the development of radiation myelopathy (RM) in rats (Abstr.). Radiother Oncol 2000;56:S15-


CHAPTER 1