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Response of pig lung to irradiation with accelerated ^{12}C-ions

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Abstract The response of pig lungs to irradiation with ^{12}C-ions was assessed in two experiments to validate the procedures for heavy ion therapy planning at the Gesellschaft für Schwerionenforschung (GSI) and to explore their range of applicability. In both experiments, the target volume (spread-out Bragg peak, SOBP) was planned to be a 4 cm long cylinder with a diameter of 4 cm. Doses in the SOBP were prescribed to be equivalent to 5×4 Gy, 5×5.5 Gy and 5×7 Gy of x-rays in the first experiment, and to 5 fractions of 7 Gy and 9 Gy in the second experiment. The lung response in the first experiment was less than expected on the basis of earlier experiments with photons. Pneumonitis reaction and chronic fibrotic changes were observed outside the prescribed high-dose region. In the second experiment, the effects were more pronounced than had been expected on the basis of the first experiment. Changes were most intense in the high-dose region, but were also seen throughout the lung along the beam channel. Moreover, significant skin reactions were observed at the beam entrance site in all animals and – less pronounced – at the beam exit site in 3 of the 6 animals. In conclusion, the complex irradiation geometry of the pig lung, the changes of body weight between the two experiments, and insufficient accounting for a change in the relative biological effectiveness (RBE) computation led to substantial deviations of the observed reactions from expectations, the reasons for which could be identified in a subsequent analysis. The less pronounced lung reaction in the first experiment was due to an overestimation of RBE in a preliminary version of the algorithm for its determination. The extension of the fibrotic reaction resulted from the smear-out of the high-dose region due to density variations in tissue structures, respiratory movement, and limited positioning accuracy. The skin reactions at the entrance port reflect the different treatment geometry in the two experiments. The one unexplained observation is the mild skin reaction that was observed in the second experiment at the beam exit site.

Key words Normal tissue · Minipig · Lung · Heavy particles · Carbon ions

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Introduction

One of the most promising approaches towards improved radiation therapy is optimisation of the spatial distribution of the effective dose. Higher doses in the target region and a steep fall-off at its margins can increase tumour cure rates without enhanced normal tissue toxicity. With photon or electron radiotherapy, new conformal treatment planning and irradiation techniques allow substantial sparing of normal tissues with unreduced local control rates, or improved tumour control without an increase in normal tissue toxicity. This has been clearly demonstrated in the treatment, e.g., of tumours of the prostate [1, 2].

An alternative is the use of accelerated ions, such as protons or heavier ions, with their particular physical and biological properties. The advantage of such particle beams is a characteristic depth-dose distribution with relatively low energy deposition in the entrance channel, a marked dose maximum (Bragg peak) towards the end of the particle track, and a steep fall-off beyond the peak. The application of varying beam energies can provide a spread-out Bragg peak (SOBP) of chosen dimensions. A further essential feature of heavy ions – such as the carbon ions (^{12}C) that are presently utilised at Gesellschaft für Schwerionenforschung (GSI) – is the increase of the relative biological effectiveness (RBE) with depth.

Clinical pilot studies of heavy ion radiotherapy at the Lawrence Berkeley Laboratory [3] and at the HIMAC facility in Chiba, Japan [4], have shown encouraging results. Work towards an experimental heavy ion radiotherapy unit at GSI in Darmstadt was initiated in 1993. It uses beams of the heavy ion synchrotron SIS with a maximum ion energy for therapy of about 430 MeV/u [5, 6]. In contrast to earlier facilities, where passive shaping methods are used, an active beam delivery system (raster scan) is used at GSI, which permits extremely tumour-conformal treatment.

A prerequisite for the clinical application of heavy ions in radiotherapy is a reliable method for the estimation of the biological effect, both in the target volume and in surrounding structures. The critical part is the quantification of the RBE that changes substantially with particle energies and with absorbed dose. This quantification is based on a biophysical model, which combines information from the photon dose-response curve, the microscopic energy-deposition pattern in the ion tracks, and the size of the cell nucleus as a critical target [7]. The model has been tested in a number of in vitro studies [6, 8], but only limited information is available from animal experiments on tumours and normal tissues [9, 10, 11]. The present study was, accordingly, initiated to supply such information.

Lung irradiation was chosen to explore the predictability of the biological effectiveness and the technique for heavy ion dose planning in a geometry that is particularly complex but also particularly suitable for a detailed analysis of the results. The size of the pig lung allows for defined irradiation of partial volumes, and the response of the lung and its spatial distribution can readily be assessed in longitudinal studies by computed tomography (CT). The lung is also a model of interest because it displays a substantial fractionation effect with regard to late fibrotic changes.

On the other hand, lung irradiation is particularly difficult because of the very low lung density. Any small range uncertainty in water-equivalent tissue translates into an approximately five-fold increased uncertainty in lung tissue due to its low density. For this reason the treatment of lung tumours is not envisaged in the present phase of the GSI project. However, the lung model has been chosen because of the advantages mentioned above and with the intent to identify critical issues more readily when the dose planning methodology is applied outside its normal range.

Two experimental series were initiated. In the first one, lung irradiation was performed in 6 animals with a follow-up of 9 months. In this series, skin irradiation with carbon ions (plateau region of the beam) or x-rays was performed in the same animals in addition to the lung treatment. The results of this skin treatment study have been reported elsewhere [11]. In the second series, where only lung irradiation was performed, doses, irradiation technique and position of the planned SOBP were adjusted according to the results of the first experimental series. Moreover, improved techniques were applied for animal positioning.

Materials and methods

All experiments were carried out with permission of the 'Regierungspräsidium Dresden' (file number 75-9168.41-1.4/94) according to the current animal welfare legislation.

Animals and housing

Six pigs of the Mini-Lewe strain aged about 12 months, weighing 50–60 kg, were used in the first experiment. In the second series, six minipigs of the same strain, but with a substantially higher average body weight of 80–100 kg were used. The animals were kept under standard conditions in the animal house of the University Hospital Dresden. In each experiment all six animals were housed in one box of 10.5 m^2.

For irradiation, the pigs were transported to GSI, Darmstadt, where they were housed during the time of treatment under conditions similar to those in Dresden.

Treatment planning and irradiation

Prior to irradiation, lung CT scans were performed in Dresden. For this, sedation of the animals was induced by azaperone at a dose of 3 mg/kg (intramuscularly), followed by premedication before anaesthesia by combined intramuscular injection of atropine (0.02 mg/kg) and diazepam (0.5 mg/kg). For anaesthesia ketamine (50 mg/kg) and xylazine (2 mg/kg) were administered intramuscularly. In experiment 1, the animals were fixed in a

lateral position on their left side. In experiment 2, they were fixed in a supine position. Individually designed vacuum pillows were utilised in the procedure. The whole lung of the animals was scanned in 10 mm consecutive sections, and the target region in 3 mm sections with the 'abdomen' kernel. The centre of the SOBP was indicated in printouts, and the data were then transferred to Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, for treatment planning.

All lung fields were treated with 5 equal fractions in 5 days by carbon ions at the heavy ion synchrotron SIS at GSI, Darmstadt. Animals were transported from Dresden to Darmstadt 1 day before the first fraction. Prior to irradiation, sedation and premedication was done as described above. Anaesthesia was initiated by intramuscular injection of ketamine (50 mg/kg) and xylazine (2 mg/kg), and maintained by intramuscular injection of ketamine 10–15 mg/kg after 30–45 min. The individualised vacuum pillows used for CT imaging were also used for the reproducible lateral (experiment 1) or supine positioning (experiment 2) of the pigs.

In the first experiment, the SOBP was planned as a cylinder, 4 cm long and 4 cm wide, centrally located in the right lung, with irradiation from a ventral field. The distance of the SOBP region from the lateral pleura was at least 2 cm. The position of a typical treatment field in this series is shown in Fig. 1A. In the second experiment, a SOBP of the same size was planned in the centre of the left lung, with treatment from a lateral field through the right lung (Fig. 1B). In this second series, a minimum distance between the SOBP and the lateral pleura of 4 cm was specified. Prescribed doses per fraction in the SOBP of the first experiment were 4.0 Gy-equivalent (Gye), 5.5 Gye and 7.0 Gye, in two animals each[1]. The resulting absorbed doses per fraction, averaged over the SOBP region, were roughly 1.5 Gy, 1.7 Gy, 2.6 Gy, 2.8 Gy, 3.9 Gy and 4.0 Gy, in animals 1 to 6, respectively. In the second experiment, prescribed SOBP doses per fraction were 7 Gye (2 animals) – in order to allow for dose overlap with experiment 1 – and 9 Gye (4 animals). The corresponding absorbed doses were 5.2 Gy and 7.5 Gy in the 7 Gye and 9 Gye group, respectively.

In experiment 1, RBE and dose calculations were based on a preliminary version and in experiment 2 on an improved version of the model established by Scholz et al. [7]. In this latter version of the model, the RBE values are – depending on the dose level, position in the field and the tissue under consideration – reduced by 5%–20% against the preliminary version. This implies that the absorbed doses were correspondingly increased in the second experiment. The differences are most pronounced in the Bragg-peak region.

The specific energy of the beam was varied between 212 and 283 MeV/u in experiment 1 and between 258 and 337 MeV/u in experiment 2. The corresponding average penetration depths (water equivalent) were 95–154 mm and 132–206 mm.

The heavy ion irradiation in the first experiment was performed in the experimental area at SIS (Cave A), where a digital range shifter was used for range variation of the beam. The experimental set-up and dosimetry were done as described by Zacharias et al. [11].

Irradiation in the second experiment was performed in the patient treatment room (Cave M of SIS), as described

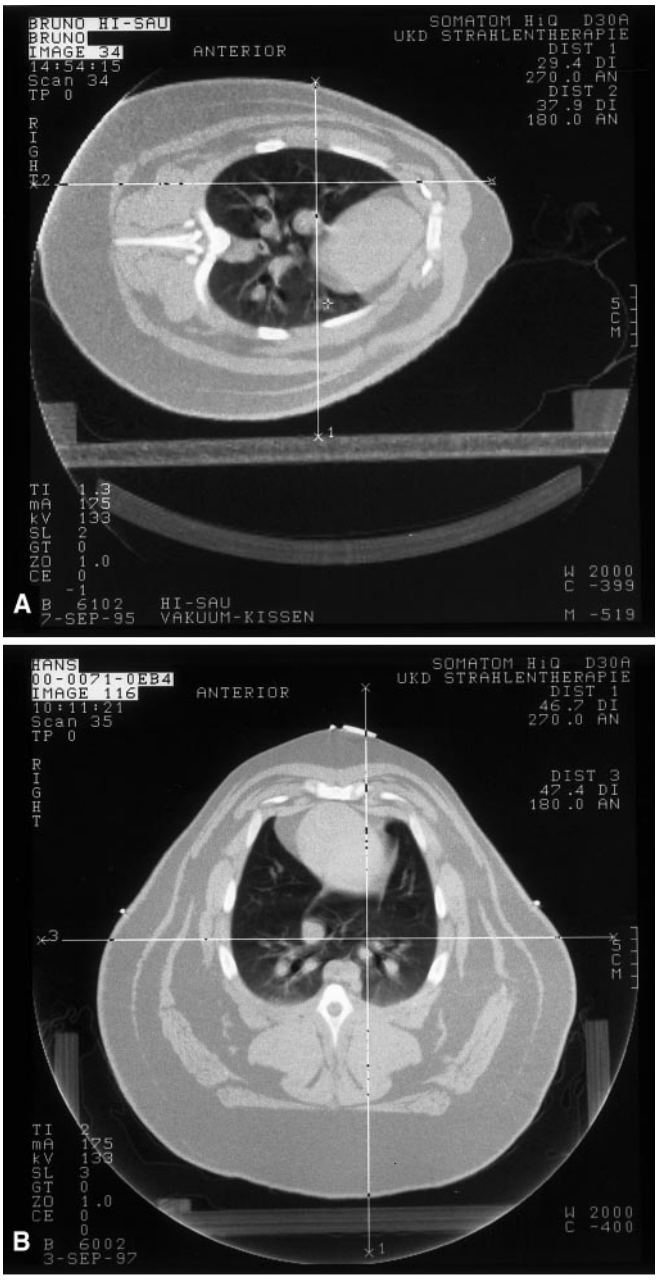

Fig. 1A, B Typical examples of treatment plans in experiment 1 (**A** ventral beam, spread-out Bragg peak, SOBP, in the right lung, animal placed on its left side) and in experiment 2 (**B** lateral beam from the right side, SOBP in the left lung, animal in supine position)

[1] The x-ray dose, D_x, that produces the same tissue effect as the heavy ion dose, D_I, is termed the x-ray equivalent dose. To avoid confusion, the symbol Gye is used as a special notation of the unit gray when related to this quantity. The symbol Gy is employed only with the absorbed dose of the specified radiation.

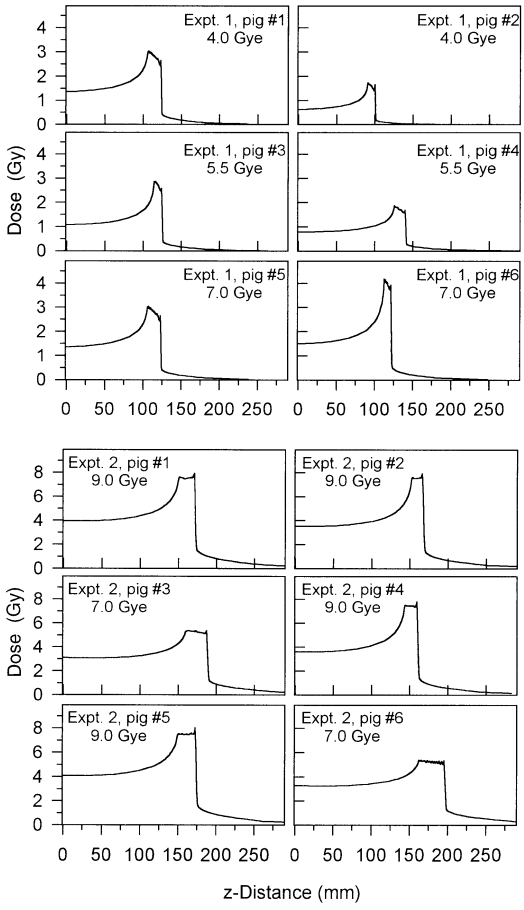

Fig. 2 Physical dose profiles along the beam channel (*z*-direction) for each individual animal in experiment 1 (*upper panel*) and in experiment 2 (*lower panel*)

recently [5]. Energy variation of the beam was performed by changed accelerator settings. On-line monitoring of the beam during irradiation [12] revealed that the lateral deviation of the delivered irradiation pattern was less than 1 mm. In addition, dose integration by means of ionisation chambers at the beam entrance side of the animals showed no deviation larger than 1.5% over the complete course of the experiment. An assessment of the three-dimensional dose distribution in a water phantom showed, as the most pronounced deviation, an underdosage of less than 10% near the end of the extended Bragg peak, which runs counter to the observed overdosage in the experiments. The dose profiles along the central beam axis are illustrated for the individual animals as a function of the water-equivalent penetration depth in Fig. 2.

Endpoints and data analysis

During the follow-up period of 9 months, all animals were submitted to clinical examination on a regular basis. The follow-up included CT scans at 6 weeks, 3 months and 9 months post-irradiation. Each of these procedures included a routine scan with 10-mm-thick sections in standard mode followed by a high-resolution scan (2-mm-thick sections, ultra-high mode) and was accompanied by a detailed clinical examination. Skin reactions in experiment 2 were scored by 3 experienced investigators who had already been involved in the definitive skin irradiation part of experiment 1. Skin reactions at the time of the CT scans were recorded according to the scoring system used in previous studies [11].

Autopsy was performed at 9 months in experiment 1. In experiment 2, due to a severe impairment in their general condition, 3 animals of the 9 Gye group were killed and underwent autopsy on days 51, 55 and 60 after the first fraction. In these animals, five samples of lung tissue along the beam channel were taken for histological examination, according to the protocol used at 9 months (see below). The remaining 3 animals of experiment 2 were killed 9 months after treatment.

Semi-quantitative radiological analysis was performed of the CT scans. The symptoms which were scored included an increased density signal, indicating thickening of alveolar septa, interstitial oedema and alveolar exudation associated with pneumonitis (6 weeks) or late fibrotic changes, in combination with shrinking or bronchiectatic processes. Autopsy findings are mainly based on fibrotic indurations. For histological assessment of the lung response, 5 tissue samples along the beam channel were taken in each of the animals: at a lateral, an intermediate, and a medial position in the right lung and at a lateral and a medial position in the left lung. The medial biopsy of the left lung corresponded to the SOBP region. The histological endpoints scored were alveolitis, oedema, hyperaemia of the alveolar septa, number of alveolar macrophages, hyaline membranes, denudation, and atelectasia.

Results

Experiment 1

As stated, CT scans were performed at 6 weeks, 3 and 9 months post-irradiation. The results of the semi-quantitative radiological analysis are shown in Table 1.

All data are compared to a historical control group which received percutaneous x-irradiation to a partial trapezoidal volume of about 500 cm^3 in the left lung. X-ray doses of 5×4 Gy are known to induce a slight to intermediate response, and 5×7 Gy a pronounced radiological response after 6 weeks [13]. After carbon ion irradiation, no response was observed after 5×4 Gye, and only a slight response after 5×5.5 Gye at 6 weeks. At 9 months after x-irradiation, pronounced late reactions are expected after 5×7 Gy, while slight to intermediate changes occur even after 5×4 Gy [13]. In the carbon ion study, no changes were seen at doses of 5×4 Gye and 5×5.5 Gye, and only an intermediate response occurred in the two animals treated with 5×7 Gye. In this context it must, of course, be noted that the target volume in the

Table 1 Intensity of lung reactions in radiological analysis of computed tomography (CT) scans at 6 weeks, 3 months and 9 months and intensity of autopsy findings at 9 months (experiment 1) or between days 51 and 60 in three animals of experiment 2. Scores indicate: – none, ? questionable, + slight, ++ intermediate, +++ pronounced, *n.d.* not done

Endpoint	Dose per fraction (Gye)			
Time post-irradiation	4.0	5.5	7.0	9.0
CT scans				
6 weeks				
Experiment 1	–	+	+++	n.d.
Experiment 2	n.d.	n.d.	+++	+++
3 months				
Experiment 1	–	?	++	n.d.
Experiment 2	n.d.	n.d.	+++	+++
9 months				
Experiment 1	–	–	++	n.d.
Experiment 2	n.d.	n.d.	++	+++
Autopsy[a]				
Experiment 1 (9 months)	+/–	+/++	++/++	n.d.
Experiment 2 (days 51/55/60)			–	+++/+++/+++
Experiment 2 (9 months)			++/+++	+++
CT expected[b]				
3 weeks	–	+	++	n.d.
3 months	+–++	++	n.d.	n.d.
9 months	+ – ++	+++	+++	+++

[a] Autopsy scores represent findings in the individual animals

[b] Data are based on a historical x-irradiated control group [13]

carbon ion exposures was smaller by about a factor of 10 than in the reference x-ray exposures.

The most intense changes were observed in the planned SOBP region of the lung, with some smear-out at the proximal margin, while the side margins of the beam channel were well-defined. No changes were seen in the entrance channel of the carbon ion beam. However, a significant increase in the signal density was also found distal from the SOBP region, i.e. in the exit part of the beam behind the SOBP, which extended to the lateral pleura.

Autopsy at 9 months revealed slight changes in one of the two animals of the low-dose group, slight to intermediate changes in the 5×5.5 Gye group, and intermediate changes in both animals of the high-dose group (Table 1). The spatial distribution of fibrotic changes confirmed the radiological findings. Fibrotic induration was found in the SOBP region. In the entrance channel of the beam, in front of the SOBP, the lung tissue appeared normal. However, the fibrotic response was not restricted to the area of the planned SOBP, but extended behind it; in the high-dose group it reached the pleura. Also, in the high-dose group, macroscopic changes were found on the lung surface at the beam exit site. No histological examinations of lung tissue were performed in these pigs.

In this experiment, no skin reactions were observed at the entrance or at the exit position of the carbon ion beam for lung irradiation.

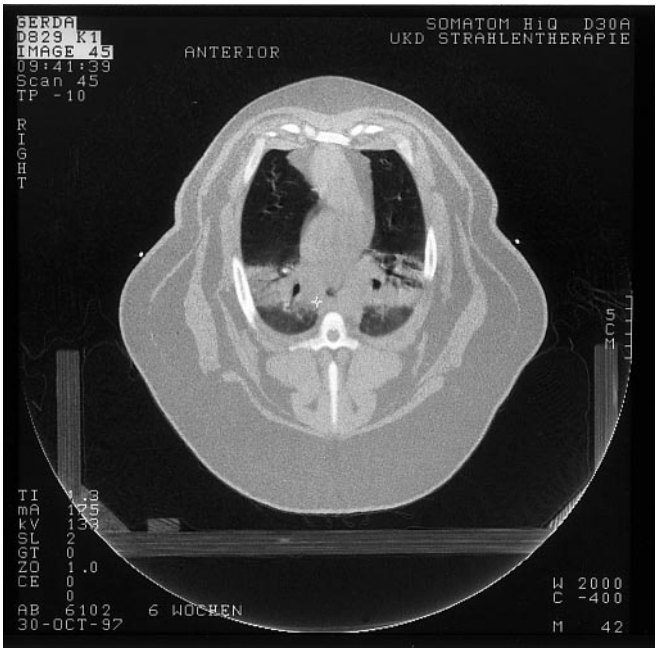

Fig. 3 The radiological response (experiment 2) assessed in CT scans at 6 weeks after the first treatment with a prescribed dose of 9 Gye to the SOBP. Marked changes are found throughout the beam channel, and no distinct region with concentration of the response (SOBP region) can be identified

Experiment 2

CT imaging at 6 weeks after irradiation revealed pronounced radiological changes in all 6 animals. These changes extended throughout the channel of the carbon ion beam and did not display a clear-cut restriction to the planned SOBP region. As in the first experiment, the side margins of the channel were well defined. A typical example of the radiological response at 6 weeks is shown in Fig. 3.

Clinical examination at 6 weeks revealed a significant skin reaction at the beam entrance location. Epilation

was observed in all animals. The response scores were 3.0 (intense erythema, dry desquamation) in both animals with 5×7 Gye to the SOBP region, while in the four animals given 5×9 Gye, two displayed a score of 3.0 (dry desquamation) and the other two 3.5 (maximum erythema).

Less intense but still significant skin reactions were observed at the beam exit site. The scores in the 5×7 Gye animals were 1.5 (mild erythema) and 2.5 (pronounced erythema); mild erythema (grade 1.5) was also observed in one of the four pigs treated with 5×9 Gye.

At 6 weeks post-treatment, i.e. at the time of the first CT scans, all pigs decreased their food intake and substantially lost body weight. In view of the severely impaired general condition, 3 of the 6 animals, all of the 5×9 Gye group, were killed on days 51, 55 and 60, respectively. Autopsy revealed a deep oesophageal ulcer which was covered by a pseudomembrane, in association with intra- to almost transmural necrosis in all 3 animals, with a reactive mediastinitis in adjacent regions. The oesophageal ulcers were limited to the posterior wall of the oesophagus, with a sharp margin.

The pathological findings in the lung corroborated the CT diagnosis. Major changes were oedema and induration. In all 3 animals, lung reactions were pronounced at the lung surface both at the entrance and the exit site of the beam. In cross-sections, changes were found throughout the beam channel, with no clearly recognisable maximum at the SOBP region.

Similarly, histological changes were found in all 5 histological samples of each of the animals in the entrance as well as in the exit channel. Predominant findings were oedema, hyperaemia, an increased number of alveolar macrophages and alveolar denudation. In the animal killed on day 60, a marked fibrosis component of the alveolitic processes was observed.

The remaining 3 animals did not show significant clinical symptoms. CT scans at 3 and 9 months confirmed the 6-week findings: Pronounced radiological changes were found in all animals along the beam channel. However, at 9 months a more intense reaction was seen in the specified high- dose region.

With regard to skin reactions, no significant response was scored at 3 months. At 9 months, however, a slight induration was observed at the entrance location of the beam.

Autopsy at 9 months after treatment confirmed the radiological results. In all animals, fibrotic indurations were found along the beam channel, with somewhat increased intensity at the SOBP position. Similarly, histological examination of the lung tissue revealed a fibrotic response in all 5 samples along the beam channel; in the medial part of the left lung, i.e. in the region of the specified SOBP, some foci of enhanced reaction were observed.

Discussion

A clinical study of the application of heavy ions for radiation therapy – initially only of head and neck tumours – has been initiated at the GSI. For the therapy planning complex algorithms are required that account for, among other factors, inhomogeneities of tissue density and the energy and dose dependent biological efficiency of the particle beams. The correction for density inhomogeneities – and thus the correct position of the extended SOBP in the specified target region – is determined by the physical properties (energy-range relation) of the particles which are well known. The correct dose planning is a more complex issue, since it depends on the less reliably known biological response characteristics of the tissue under consideration. It is, therefore, a critical issue to validate the biological models not only by dosimetric checks and by cell culture studies but also by animal studies with a variety of clinically relevant biological endpoints. The present investigations must be seen in this context. The radiation response of the lung is a suitable endpoint in an animal study, because it can be studied non-invasively in a large animal such as the pig, which permits the determination of the topographical distribution of the biologically effective dose. On the other hand, it offers – more than other experimental models – difficult obstacles to treatment planning. The difficulties are predominantly due to the great variations of tissue densities in the beam channel, including skin, ribs, fatty tissue, lung tissue, mediastinal structures, and air.

In the present study, the lung response to carbon ion irradiation was investigated in two experimental series, each on 6 minipigs. The endpoints of primary interest were radiological changes that reflected the pneumonitis response at 6 weeks after treatment, and lung fibroses at 3 months and at 9 months. Autopsy was performed at 9 months. In the first experiment, the doses from the carbon ion beam in three dose groups were chosen on the basis of historical control data [13, 14] so that minor, intermediate and pronounced changes would result, both in the repeated radiological assessment and at autopsy. To this purpose, a recently developed biophysical model [15, 16] was applied.

The first experimental series resulted in lung responses that were significantly less than expected. From clinical and experimental experience, it is known that with regard to structural changes in the lung, the volume effect is minor. It is, thus, unlikely that much of the difference is due to the reduced effect of a dose given to a 50 cm^3 volume, rather than the 500 cm^3 volume in the earlier reference experiments with photons. The result thus indicates that the efficiency of the carbon beam was overestimated in the biophysical model that was employed.

A second unexpected observation was a spatial distribution of the response that extended beyond the planned high-dose region. Marked reactions were found in the beam exit channel behind the SOBP, extending to the lat-

eral pleura. Several factors may have contributed to the unexpected spatial distribution of the fibrotic reactions. The first, and probably major, point is that positioning inaccuracies as well as respiratory movement can influence the dose distributions substantially. In line with this consideration, the lung response displayed sharp side margins, indicating that the lung was in the same position for each fraction, but due to the finite width of the beam and of the CT slices, positioning errors of only 3–5 mm can result in considerable range variations. A second relevant point is that the extended region of response in the lung may be due to an accompanying pleural reaction outside the treatment volume, or at least beyond the high-dose area. Such reactions are frequently found in radiotherapy patients with lung irradiation fields close to the pleura. The minimum distance between the SOBP region and pleura in the first experiment was 2 cm, which – according to clinical experience – should be just about sufficient to avoid reactive pleural changes. To obtain further information with regard to possible secondary, reactive pleural responses, this distance was increased to 4 cm in the second experiment.

To account for both the underestimation of the biological effect in the SOBP region and the unexpected topographical distribution of the response, several methodological changes were introduced in the second experiment. The dose was increased so that, based on the results of the first series, a full response in the target region should have been achieved; furthermore, to obtain overlap with the first experiment, a dose group with 5×7 Gye in the SOBP was added. The irradiation technique was substantially changed. The animals, which were substantially larger in the second experiment, were treated in a supine position to attain more precise positioning. Furthermore, the planned SOBP was placed in the centre of the right lung, with a minimum distance of 4 cm between the target volume and pleura. Finally, an improved version of the biophysical model [7] was employed for the calculation of the effective doses to the lung; this resulted in a further increase of absorbed doses in the second experiment.

The severity of the lung reactions observed in the second experiment agreed with the prediction based on the historical control experiment. However, a considerable smear-out of the reaction was again observed. While this conflicted with initial expectations, it was readily understood in terms of detailed dosimetric considerations, which make it clear that it is impossible to plan the transmission of the ions through the irregular structure of the thoracic wall adequately. High-energy ions may or may not pass through a rib, which then results in very large range differences in the lung. The effect is apparent in both experiments. The dense structures, and especially the ribs, that were included in the entrance channel were responsible for the smear-out, and these were amplified by the breathing excursions of the thorax. The larger size of the animals in the second experiment compounded these problems further.

A major complication in the second experiment was unrelated to the purposes of the investigation. The shift of the target volume from the left to the right lung was associated with an incidental and unintended inclusion of mediastinal structures in the region of increasing dose in front of the SOBP proper. As a consequence, the irradiation caused severe acute toxicity in the oesophagus, which necessitated killing 3 animals due to intra/transmural oesophageal ulceration. Detailed literature data on oesophageal radiosensitivity are scarce. In mice, acute single LD_{50} values for this endpoint of 25–32 Gy and higher have been reported for sparsely ionising radiation [17, 18, 19]. After irradiation with 10 fractions, the acute LD_{50} increased to 57.5–71 Gy [19, 20]. In dogs, intraoperative irradiation with single doses of 30 Gy was not lethal [21]. Similarly, after percutaneous irradiation of canine oesophagus with doses up to 52 Gy in 4 fractions, no acute deaths were reported [22]. In a recent study in dog lungs [23], where the entire thoracic oesophagus was irradiated with doses between 45 and 72 Gy in 1.5-Gy fractions, 16 of 128 dogs developed clinical signs of oesophagitis; 3 of them had to be killed. The authors concluded a ED_{50} for oesophagitis of 67 Gy (95%CL 61.5 Gy, 79.7 Gy).

Assuming an α/β-ratio for the acute lethal oesophageal response of 10 Gy, a single-dose LD_{50} of 25 or 30 Gy is equivalent to 46 or 56 Gy given in 5 fractions. Similarly, a LD_{50} of 60 or 70 Gy in 10 fractions is equivalent to 49 or 56 Gy in 5 fractions. On the basis of these considerations, the doses to the oesophagus in the second experiment, which were planned to be less than 45 Gye, would have been expected to be still tolerable. Retrospective analysis of the effective dose distribution, however, has indicated that the effective doses in the oesophagus were in the range of 45–50 Gye, which is close to the estimated LD_{50} doses, and can explain the observed complications.

In contrast to the first experiment, radiation-induced changes of the skin were observed in the second experiment not only in the entrance channel of the beam, but also in the exit channel. These reactions were scored on day 49 after the first fraction, which is within the period of maximal response in skin irradiation experiments in the same minipig strain [11].

As a separate component of the first experiment, the skin treatment with carbon ions (plateau region) was compared to the effects of 200-kV x-rays [11]. This permits an estimate of the effective dose that could explain the skin damage observed in the second experiment. The skin experiment in the first series was meant to produce no response, or intermediate and full skin response in the three dose groups of both the x-ray and the carbon ion arm. Six skin fields were used for each dose group.

The dose-effect curves from the skin experiment can be used to determine the doses that are required to explain the different response scores. The procedure is illustrated in Fig. 4 for the entrance channel skin response in the animals with 9 Gye specified in the SOBP. Skin entrance scores of 3.0, 3.5 and 4.0 were observed in 4/4,

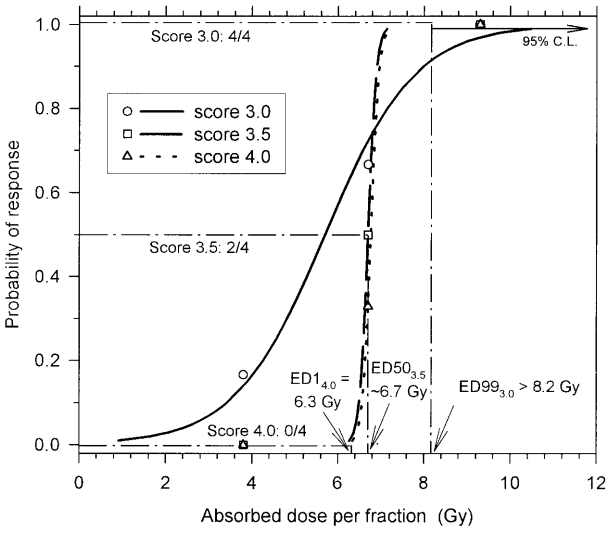

Fig. 4 The method applied to estimate the effective x-ray-equivalent doses in the entrance and exit channels of the heavy ion beam in the second experiment. The detailed procedure is given in the text

2/4 and 0/4 animals. The ED_{50} for score 3.0 was estimated to be 5×5.7 Gy, the ED_{10} for score 3.5 was deduced to be 5×6.5 Gy. As all 6 animals reached score 3.0, this implies that the effective dose was certainly not less than the ED_{50} for score 3.0. Thus, the skin dose must have been in excess of 5×5.7 Gye. That 2 of 6 animals reached score 3.5 implies that the effective dose must have been at least roughly equal to the ED*10* for score 3.5. Thus, it must have been not less than about 5×6.5 Gye.

The results for both dose groups obtained in this way for the reaction in the entrance and in the exit channel are summarised in Table 2. The general conclusion from this analysis is that in the 5×7 Gye group, effective doses in the entrance and exit channel must have been of the order of 5×6.5 Gye and 5×3.4 to 5×3.9 Gye, respectively. The corresponding estimates for the 5×9 Gye group are 5×6.7 Gye for the beam entrance and below 5×3.4 Gye for the exit site.

Thus, the skin responses at the beam entrance were substantially higher than had been expected. In addition, a marked lung response was induced in the irradiated volume in front of the SOBP volume in the second experiment. Two factors appear to have contributed to this increase in the biologically effective dose of the carbon ion beam in the entrance plateau region. First, the treatment geometry was changed, which resulted in a greater fraction of dense structures (mediastinum) in the entrance part of the channel which, in consequence, required higher beam energies. The higher beam energy reduced the dose ratio between the peak and plateau. In the first experiment the ratio was 2.6:1 for the animals with a SOBP dose of 7 Gye; the ratio in the present experiment was calculated to be no more than 1.6:1. Second, the algorithm applied for RBE calculation was modified in a way that corresponded to a decrease in RBE in comparison to the earlier formulation. Hence, the absorbed dose was increased for the 5×7 Gye group, from roughly 5×4 Gy to about 5×5 Gy in the SOBP**.**

Table 2 Estimates of skin doses in the entrance and exit channels of the carbon beam in the two dose groups. The calculations are based on the results of a previous experiment, where definitive skin irradiation was carried out with 200-kV x-rays or carbon ions (plateau region of the beam). The results of this experiment are published elsewhere [11]. The method used for dose estimation is explained in the text and illustrated in Fig. 3. Dose estimates were made based on the effect of x-irradiation. Numbers in parentheses indicate the error of the estimate; types of errors, i.e. standard deviation (σ) or lower or upper 95% confidence limits (*lCL, uCL*) are indicated. In some cases (–) no dose-effect curve could be established because the score was reached in all of the fields irradiated

SOBP dose(Gye)	Score	Frequency	X-ray dose(Gy)
Entrance channel			
7	3.0	2/2	10.5 (lCL 8.2)
	3.5	0/2	<6.3
	4.0	0/2	<6.3
9	3.0	4/4	10.5 (lCL 8.2)
	3.5	2/4	6.7 (σ 0.1)
	4.0	0/4	<6.3
Exit channel			
7	1.5	2/2	–
	2.0	1/2	3.9 (σ 0.1)
	2.5	1/2	3.9 (σ 0.1)
	3.0	0/2	<0.9 (uCL 3.4)
9	1.0	4/4	–
	1.5	1/4	–
	2.0	0/4	<3.4

As a consequence of the changes in the experimental methods, the absorbed dose to the skin in the entrance channel was increased in the 5×7 Gye group, from about 1.7 Gy per fraction in the first experiment to about 3.1 Gy per fraction in the second series. The absorbed dose in the entrance channel in the 5×9 Gye group was about 5×3.8 Gy (Fig. 2). The absorbed doses of 3.1 Gy and 3.8 Gy per fraction correspond to effective doses of 4.3 Gye and 4.9 Gye per fraction. The observed skin reactions suggest, in fact, an effective dose that was >30% higher.

The reasons for the skin reaction at the exit site of the carbon beam remain unclear. The carbon ion dose at a distance >10 cm from the distal margin of the SOBP should be negligible. Some contribution to the effective dose may result from charged fragments; however, these cannot fully account for the skin reactions that have been observed. No substantial contribution is expected from neutrons. A target dose of 60 Gy in a 5×5×5 cm^3 cube is associated with a neutron dose of 0.8 Sv. A review of the treatment plans of the individual animals did not reveal any significant errors. Yet the observed skin reactions at the beam exit suggest a dose about half as large as in the SOBP. There is no indication from other in vitro or in vivo experiments which would suggest the unexpectedly high RBE and could explain the observed reaction.

Conclusions

The dose planning with high energy carbon beams encounters great complexities if structures of widely differing densities are included in the beam channel, and particularly if the resulting problems are compounded by movement, e.g. due to respiratory excursions. The present experimental investigations included the added difficulties which arise from the problems of precise and reproducible fixation of the test animals, and also from the fact that the size of the animals was substantially larger in the second experimental series. The largely unexpected results confirm the strategy that the heavy ion therapy program at the GSI will – at least in its initial phase – be restricted to the much simpler geometry that arises in the treatment of head and neck tumours.

On the other hand, the experiments have provided a valuable learning process which helped to focus on critical steps in the dose-planning procedures, and which demonstrated that a critical re-evaluation was able to explain most of the features of the biological responses observed. The severity of the lung reaction in the second experiment was in agreement with the response expected from historical control data. The lower than expected lung reaction in the first experiment was due to an overestimation of RBE in the preliminary version of the biophysical model used in the treatment planning procedure. The observed smear-out of the fibrotic reaction can be attributed to the inclusion of structures of varying density (ribs, intercostal spaces) in the entrance channel and their movement due to respiratory excursions.

The different treatment geometry in the second experiment, associated with a considerably higher dose in the entrance channel, was found to be the major cause for the skin reactions observed in this experiment. However, the remaining dose difference of 30% demonstrates a clear discrepancy between the predictions from biophysical modelling and observed biological effects. This discrepancy, however, has to be judged in the light of the uncertainties of the model prediction, which are of the order of 15%, as well as the uncertainties of the experimental procedure for statistical reasons. There is no explanation, at the moment, for the skin reactions at the exit side, which were more pronounced than expected from the absorbed doses and the estimated effective doses at this position. Both these skin responses in the entrance and exit channels require further investigation.

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