lung for the mid- and distal esophageal cancer using four-dimensional CT (4D-CT).
Materials and Methods: Eight patients with middle and distal esophageal carcinoma planned for three-dimensional conformal radiotherapy underwent respiration-synchronized 4D-CT simulation during free breathing. All image sets were registered with the reference image (T0 phase),and the GTV, the dome of diaphragm, lung, heart and skin markers were delineated on CT images of the ten respiratory phase. The position of GTV, dome of the diaphragm, lung, heart and skin markers were identified in all 4D-CT phases, and the volume of GTV, lung and heart were also achieved.
Results: The primary tumour motion was maximal in the superioinferior direction. The correlation between the primary tumour and the dome of diaphragm, lung, heart was best in superioinferior direction, the mediolateral GTV displacement correlated with the right lung and heart ($r = 0.709,\ 0.800$; $P = 0.022,\ 0.005$). There was no relationship between the GTV displacement and the skin markers. The GTV volume was correlated well with the lung volume ($r_{\text{GTV-left lung}} = 0.745$, $P = 0.013$; $r_{\text{GTV-right lung}} = -0.736$, $P = 0.015$), but the correlation was not significant with the heart ($r = -0.138$, $P = 0.705$).
Conclusion: Heartbeat and expansion of the chest wall correlated with displacement of primary carcinoma of the middle and distal oesophagus; the external surrogate can not verify the GTV displacement of primary esophageal cancer during free breathing.

2029 POSTER

Biological Quality Assurance of Carbon-ion Beam Irradiation at Spread-out Bragg Peak (SOBP) Beams

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Background: We have to compare and confirm relative biological effectiveness (RBE) of carbon ion beam among several facilities using standard and common biological quality assurance method. For the quality control of heavy particle beam therapy machines, validation of the stability of the physical dose is important, but validation of the biological effects of each machine is also necessary. For this purpose, the establishment of a standard method for validation of the biological effects and RBE is desired. We will confirm new brief assessment methods using another biological endopoint of RBE values.
Materials and Methods: The SOBP was designed on the basis of the survival curve of the human salivary gland cancer cell line HSG. Reference X-ray irradiation was performed by 130kv, RX-650. Culured cells from HSG cells were irradiated at 4 points along 290 MeV per nucleon carbon ion beam, with 6 cm SOBP. Irradiated cells were immediately prepared for cell survival assay using colony formation method. The degree of this G_2 block has been reported to be dependent on the LET. This is also considered to be a cause of the marked cytocidal effect of high-LET radiation. Cell cycle distributions were analyzed by flowcytometry (FACScan) at 6, 12, 24 hours after carbon ion irradiation. We compared our data with date of other institute.
Results: RBE values of carbon ion beam were calculated from cell survival curves at the dose that would reduce cell survival to 10% (D_{10}) compared to X-ray irradiation. The RBE is higher in deeper regions, and RBE values at proximal (-25 mm), center (0 mm), distal (+25 mm), and distal end (+28 mm) of 6 cm SOBP were 1.6, 2.0, 2.4, and 3.3, respectively. The marked G_2 block at 12 hrs appeared, and the degree of G_2 block was dependent on irradiation dose and RBE. RBE value is similar to that of NIRS (National Institute of Radiological Sciences).
Conclusion: Cell survival assay and cell cycle analysis are considered to be important for biological quality assurance to assure the validation of biological effects at SOBP.

2030 POSTER

Knockdown of the Apoptosis Related Protein Survivin Leads to an Increased Radiosensitivity of Ewing Sarcoma in Vitro

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Background: Survivin is a protein of 16.5 kD and belongs to the IAPs (inhibitor of apoptosis proteins). It is overexpressed in nearly all solid tumours and leukemias. Survivin function depends on its subcellular localization: as a nuclear protein it regulates cell division whereas the transport into the cytosol changes its function to apoptosis inhibition. The huge prognostic and predictive value is described in several publications. We investigated its influence on radiation response in Ewing sarcoma, an aggressive childhood tumour with poor prognosis.
Materials and Methods: Protein expression was investigated by Western blot experiments while DNA double strand breaks (DSBs) and repair was quantified by flow cytometric determined γH2AX. Apoptosis was determined flow cytometrically by using the Annexin V test. SiRNA based knockdown experiments were done by liposomal transfection.
Results: Survivin protein was upregulated in different Ewing sarcoma cell lines in a dose dependent manner. As a result of Survivin knockdown STA-ET-1 cells show a reduced cell proliferation, an increased number of DSBs and a reduced repair. Apoptosis was increased by knockdown alone and rises further in combination with radiation injury.
Conclusions: Survivin is a radiation inducible protein in Ewing sarcoma cell lines and increases with increasing single dose. Knockdown experiments revealed its strong influence on DSB repair, cell proliferation and apoptosis and thus, underline its radioprotective function in Ewing sarcoma. Therefore, Survivin may be an important target and may open new therapeutic options to treat this aggressive childhood tumour.

2031 POSTER

Low-dose Pulsed X-ray Antitumour Efficacy at the Model of Lewis Lung Carcinoma

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Background: Radiotherapy is widely used to combat many cancers. However, high doses of radiation to reach marked therapeutic effect bring about the heavy side effects. Low-doses of continuous ionizing irradiation have been shown to be not enough efficient to treat cancer. Biological effects could be increased by using pulse-modulated radiation. The source of low-dose repetitively pulsed X-ray radiation was first developed and created at the Institute of high-current electronics (Russia). The purpose of this study is to investigate the antitumour and antimetastasis efficacy of low-dose repetitively-pulsed X-ray at the mice tumour growth model.
Materials and Methods: Solid-type of Lewis lung carcinoma was prepared by intramuscularly transplantation of 3×10^6 cells into the hind limb of C57BL/6 female mice. Cell proliferation was measured by [^{3}H]thymidine incorporation into cells DNA using liquid scintillation counting. Tumours were allowed to attain a volume of 350–750 mm^3 when irradiation was initiated. Tumour volumes were measured with calipers and a volume calculated (L+W+W/2). The metastases of the lung were counted using a stereoscopic microscope. Dose rate was 0.1−1.7 R/min, time of irradiation was 6 min approximately, absorbed dose was 4−30 mGy, pulse repetition frequency 8−19 c^{-1}.
Results: Low-dose repetitively pulsed X-ray inhibits proliferation of Lewis lung carcinoma cells in vitro at 50−60%. Effect depended on pulse repetition frequency and dose rate. The maximal effect observed at regimes: [10 c^{-1} and 0.18 R/min]; [13 c^{-1} and 1.17 r/min] and [16 c^{-1} and 0.96 R/min]. Irradiation of mice with Lewis lung carcinoma at selected regimes on day 7 and 14 after tumour transplantation leaded to statistically inhibition of tumour growth. The most efficacy regime was 13 c^{-1} (20% of inhibition), while decreasing of tumour growth at 10 and 16 c^{-1} were 13 and 10%. Same time, Index inhibition of metastasis was 30% in group [10 c^{-1} and 0.18 R/min], but there were not observed any changes compare to control in other groups. Moreover, outside necrosis (when the tumour size is too big and force through the skin) and gangrene of mice limb were 2-times lower in group [10 c^{-1} and 0.18 R/min] compare to control group, while irradiation at 13 and 16 c^{-1} leaded to increase of this indexes. So, selected regime needed to be further investigated to increased antitumour efficacy.
Conclusion: The results is evidence of availability further investigation of repetitively-pulsed low-dose X-ray antitumour effects in case of its possibility medico-biological application, especially in oncology.

2032 POSTER

Radiation-induced Microangiopathy in the Rectum Using an Animal Experimental Model

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Background: The purpose of the present study was to examine the sequential change of radiation-induced microangiopathy using an

experimental rat model of radiation proctitis, and to assess the severity of microangiopathy.
Materials and Methods: A total of 57 Wistar rats were used. 45 of the rats were exposed to selective rectal irradiation with a single fraction of 25 Gy. These rats were sacrificed at the 4th, 12th, 24th, and 37th week following the irradiation. The remaining 12 rats comprised the control group without irradiation. The microangiopathy was examined pathologically regarding the rectum in 20 mm from the anus of each rat. The absolute number of vessels was counted by microscopy. In addition, the diameter stenosis of stenosed vessel was evaluated and graded the degree from 0 to 4. The specimens of the rats, which had been sacrificed at the 10th day following irradiation in the previous study, were also examined pathologically to compare the differences between acute changes and chronic changes following irradiation.
Results: The sequential changes of radiation-induced microangiopathy were examined well. The microangiopathy was observed selectively in the arteries. The vascular endothelial damage was observed mainly due to nuclear bulging in the rats on the 10th day following irradiation. Whereas, the thickening that accompanied the fibrinoid necrosis after 4th week, and the thickening of endothelial lining was significant later. The absolute number of vessels per individual was 289.7 ($\pm$63.5), 385.8 ($\pm$60.6), 256.6 ($\pm$70.0), 282.1 ($\pm$57.1), and 141.4 ($\pm$47.5) at 4th week, 12th week, 24th week, and the 37th week following irradiation, respectively. The number of vessels was significantly smaller in the rats without irradiation than the irradiated rats and was significantly greater at the 12 weeks following irradiation than the other groups ($P < 0.05$). The degree of stenosis was evaluated in the microvessels microscopically. No significant differences were found among the groups in terms of the proportions of severe vascular stenosis. The proportions of the stenosed vessels that occupied a portion of the absolute number of the vessels were 16.0%, 10.6%, 13.3%, and 14.6% at 4th week, 12th week, 24th week, and the 37th week following irradiation, respectively.
Conclusions: We examined the sequential changes of radiation-induced microangiopathy. Our assessment strategy of microangiopathy seems to be useful to evaluate the severity of late radiation proctitis.

2033 POSTER

Radiation-induced Rectal Toxicity in Rats on Low-dose Aspirin Therapy

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Background: The purpose of the present study was to establish an animal experimental model of radiation proctitis in rats receiving antiplatelet therapy, and to examine the correlation between the administration of aspirin and the severity of radiation proctitis.
Materials and Methods: A total of 34 female Wistar rats were used. The rats were divided into five groups: aspirin 5 mg/kg/day group (ASA5; n = 10), aspirin 10 mg/kg/day group (ASA10; n = 10), aspirin 20 mg/kg/day (ASA20; n = 7), and saline group (Saline; n = 7). The rats were administered with aspirin at dose of 5, 10, 20 mg/kg or saline orally, day by day before and after irradiation. On the fifth day following the start of administration, all rats were irradiated and the tail transection bleeding time was measured. A single fraction of 25 Gy was delivered selectively for the rectum without any surgical procedures. The administration of aspirin or saline continued daily following irradiation. All rats were sacrificed at the 10th day following irradiation.
The rectal mucosal changes of each rat were evaluated macroscopically and pathologically. In the pathological examination, the severity of proctitis was described the morphological mucosal damage and the degree of inflammation in each specimen.
Results: The bleeding time was prolonged in rats receiving aspirin.
The proportion of the severe changes in macroscopic findings was 100.0%, 50.0%, 66.7% and 66.7% in the ASA5 group, the ASA10 group, the ASA20 group, and the Saline group, respectively. In the morphological mucosal damage, the propotion of the severe changes was 70.0%, 71.4%, 50.0% and 80.0% in the ASA5 group, the ASA10 group, the ASA20 group, and the Saline group, respectively.
There were no apparent correlation between the administration of aspirin and the severity of radiation proctitis in the macroscopic findings, and the morphological mucosal damage in the pathological examination.
The proportion of the severe degrees of inflammation was 90.0%, 100.0%, 16.7% and 100.0% in the ASA5 group, the ASA10 group, the ASA20 group, and the Saline group, respectively. The ASA20 group showed significantly milder inflammation than the other groups ($P < 0.05$).
Conclusions: We established an animal experimental model of radiation proctitis in rats receiving antiplatelet therapy with the use of low-dose aspirin. There were no apparent correlations between the administration of aspirin and the severity of radiation proctitis.
The influence of low-dose aspirin on radiation proctitis is presently under investigation in more detail.

2034 POSTER

Evaluation of Two Registration Strategies for Inter-patient Dose Mapping in Prostate Radiotherapy

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Purpose: Compare dose distributions from different patients is necessary to assess correlations between toxicity and organ at risk dose distribution. This comparison implies mappings in a common template. Registration methods are classically validated with spatial overlap metrics (DiceScore (DS)), which are not designed to validate dose mapping (DoM). The objective of the work was to evaluate 2 elastic registration methods by using usual and new metrics.
Methods: The study included 24 patients (pts) receiving 3D conformal radiotherapy for prostate cancer.
Registration Methods: The planning data (CT scan images, contours, dose distribution) of 23 pts were registered on the planning data of the 24th chosen as template (the most representative pt according to mutual information results). Two registration strategies initialized by CT-Scan intensity based affine registration (AR) were used:
- Iconic: a CT-Scan intensity based non-rigid FFD registration was applied to the AR results,
- Hybrid: After AR, distance maps (DiM) were computed for each delineated organ (prostate, bladder, rectum) of each pt and of the template. The CT-Scans images were then combined with the 3 organs DiMs, and an intensity based non-rigid demons registration was applied.

Eventually the elastic transformations were applied to the delineated organs and dose distribution to propagate them in the template.
3 Metrics to validate registration Methods:
- DS between two structures A and B: $DS(A,B) = 2|A \cap B|/(|A| + |B|)$.
- Relative Difference of Areas (RDA): The DVH is assumed to be conserved before (time1) and after (time2) deformation. This conservation can be evaluate by computing the distance (RDA) between normalized DVH1 and DVH2, defined on 0,Dmax
  $RDA = (\int_{[0,Dmax]}(DVH1 - DVH2)\,dx)/\max\{\int_{[0,Dmax]} DVH1\,dx, \int_{[0,Dmax]} DVH2\,dx\}$.
- Dose and Organs Overlaps (DOO): The DOO compares the propagated dose D received by the template organ A and the propagated organ B: $DOO(D,A,B) = (\int_{A \cap B} D(x)\,dx)/(\int_{A \cup B} D(x)\,dx)$.

Results: In heterogeneous dose areas, different RDA/DOO values were found for a same DS, showing the interest of the new proposed metrics. The hybrid registration method provided significantly more accurate results than the iconic one, for each organ and with each metric (t-test, $p < 0.05$).

	Median DS	Median RDA	Median DOO
Iconic	0.69	0.56	0.11
Hybrid	0.75	0.70	0.09

Conclusion: The hybrid registration method using both organs delineations and intensity provides better results than the iconic one and should be used to analyze dose distributions and toxicity from different patients.

2035 POSTER

Early Mortality After 40,670 Courses of External Beam Radiotherapy in Unselected Patients

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Background: The UK Government have recently published their strategy for cancer, aimed at improving outcomes for patients. In this, they have recommended the measurement of 30 and 90 day mortality after palliative and radical/adjuvant radiotherapy respectively. We are unaware of any published data regarding these end-points in unselected patients and hence feel this outcome measure is poorly defined.
Material and Methods: St James's Institute of Oncology (SJIO) is a regional cancer centre providing radiotherapy for the 2.7 million population