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Evaluation of Feasibility of ^{201}TlCl Scintigraphy for Monitoring Radiotherapeutic Effects

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放射線治療効果判定におけるタリウムシンチグラフィの有用性の検討

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放射線治療効果判定におけるタリウムシンチグラフィの有用性を家兎に移植したVX-2腫瘍に20及び40 Gy放射線照射し検討した．タリウムの腫瘍集積の程度は腫瘍の対側正常筋肉組織に対する集積比で評価した．40 Gy照射群はいずれの腫瘍も縮小消失したが，20 Gy照射群では照射後，増大を示すものと縮小消失を示すものがみられた．これらのうち縮小消失を示す場合には照射後7日目の集積比は，非照射群や増大群に比較して有意に低下し，その後もさらに低下を示した．20 Gy照射群では照射後7日目の腫瘍体積は照射前に比較して軽度増大していたが，タリウムの集積比が照射前に比し30%以上の低下を示した群とそれ以下の低下にとどまった群で，腫瘍体積のその後の変化をみると前者では縮小消失したのに対し，後者では増大を認めた．

腫瘍増殖能を反映すると考えられるBrdUの取り込みを検討すると40 Gy照射群の照射後7日目の腫瘍体積は照射前に比し軽度の縮小を示したのみであるが，顕著に低下しておりタリウムの集積の低下を伴っていた．

今回は20 Gyと40 Gy照射群のみの検討であるがタリウムシンチグラフィにより腫瘍体積にかかわらず照射後，比較的早期に腫瘍増殖能を評価できる可能性が示唆された．

Introduction

Evaluation of treatment efficacy in malignant tumors has been mainly performed by tumor size measurement on X-ray CT scan, US and MR imaging. However there is an inherent problem that the differentiation of the residual viable tumor from necrosis or fibrous tissue is insufficient with such imaging modalities[1]. To solve this problem, the study of positron emission tomography (PET) has recently been introduced for the evaluation and prediction of therapeutic efficacy, providing useful information regarding the disturbance of amino acid or glucose metabolism caused by the treatment[1)-3)].

On the other hand, previous basic studies demonstrated that thallium-201 chloride (^{201}TlCl), which

is available for SPECT and has been widely used for positive imaging in various tumors[4)-7)], accumulates in the tumors mainly in accordance with (Na^+-K^+) ATPase activity as related to tumor cell activity or viability[8)-11)] and that ^{201}TlCl hardly accumulates in the nonviable tumor cells[12)]. Considering these findings, ^{201}TlCl scintigraphy should be useful for assessing the changes of viability or proliferative potentiality of tumor cells and for detecting residual tumor cells after treatment, but few investigations to assess the value of ^{201}TlCl scintigraphy for this purpose has been conducted. In the present study, we conducted basic animal studies to evaluate the feasibility of using ^{201}TlCl scintigraphy for monitoring radiotherapeutic effects.

Materials and methods

The VX-2 tumor, which has been maintained in our laboratory, was implanted in the femoral muscle in total of 41 Japanese white rabbits weighting 2.5-3.5 kg. Anesthesia was administered by injecting Nembutal (pento-barbitone sodium) 50 mg kg^{-1} via the ear vein.

Radiation therapy and tumor growth curve

Irradiation was performed 7 days after the implantation, by which time the tumors had grown to between 20 and 37 mm in diameter as measured by CT. The rabbits were fixed in the supine position and the tumors were exposed to a single dose of either 20 Gy or 40 Gy irradiation at a dose rate of 97 cGy/min using a copper and aluminum filter.

The tumor size was measured on CT imaging in a time course, and the product of three principal diameters of each tumor ($\pi/6 \times$ width $\times$ length $\times$ height) was designated as tumor volume. Tumor volumes measured after irradiation were normalized at the time of treatment (1.0). In the control tumors, the relative tumor volumes were calculated compared to the volume size (1.0) at 7 days after the implantation. The effects of irradiation on tumor growth were evaluated by tumor growth curves. Nine rabbits received 20 Gy irradiation and 5 received 40 Gy, and 8 nonirradiated controls were used in this dose-response growth study.

^{201}TlCl scintigraphy

The rabbits were fixed in the supine position over a collimator and detector, and the lower limbs of the rabbits were fixed firmly to prevent movement. Static images taken during 5-10 minutes after the injection of 200-300 MBq ^{201}TlCl administered intravenously via the ear vein were obtained using a wide-field view camera, with a 20% window centered over 69-80 keV. In addition, in 4 various sized control tumors, in 4 tumors which were 20 Gy-irradiated (2 were 7 days and 2 were 14 days after irradiation) and in 3 tumors which were 40 Gy-irradiated (2 were 7 days and 1 was 14 days after irradiation), sequential images were obtained at 1 frame min^{-1} immediately after the injection for 60 minutes to investigate the ^{201}TlCl kinetics of tumor accumulation. In addition, static images 1 and 60 minutes after the injection were also obtained for comparison of both images.

Quantitative evaluation of ^{201}TlCl uptake in the tumor was expressed as the count ratio of the most intensive uptake site within the tumor over the contralateral muscle region (tumor uptake ratio), obtained from square regions of interest (ROIs) placed over both sites. When the distribution of ^{201}TlCl uptake was seen peripherally in cases of relatively large tumors with central necrosis, the ROIs were set in these peripheral portions of the tumor. The changes of the tumor uptake ratio after irradiation were monitored, stimultaneously with the obtaining of the tumor growth curve.

Histological study

Histological observation was conducted in 17 tumors by hematoxylin and eosin-stained specimens and the results were compared with the distribution of ^{201}TlCl in the static image. Those contained various sized control tumors, 3 tumors which were 20 Gy-irradiated and 7 tumors which were 40 Gy-irradiated. Moreover, a histological survey of the 3 irradiated rabbit thigh tissues, was made and showed the disappearance of palpable tumors and ^{201}TlCl accumulation 35 days after irradiation. One of them was from a tumor-bearing thigh after 20 Gy-irradiation and the others were after 40 Gy-irradiation.

Anti-BrdU MoAb staining

To evaluate tumor proliferative potentiality after irradiation, tumor uptake of bromodeoxyuridine (BrdU) was investigated by anti-BrdU monoclonal antibody (MoAb) staining, and compared among 6 controls (7 days after implantation) and 7 tumors which were 40-Gy irradiated (7 days after irradiation). The mean tumor volume of the controls and the irradiated group examined in this study were 27.8×10^3mm^3 and 32.7×10^3mm^3, respectively. The relationships between the staining of anti-BrdU MoAb and tumor uptake ratio of ^{201}TlCl were also investigated. BrdU is an analog of uridine but is incorporated specifically into DNA in place of thymidine. Anti-BrdU MoAb can be then used to identify those cells which were undergoing DNA synthesis during exposure to BrdU. Thirty minutes after the injection of 25 mg/kg of BrdU administered intravenously via the ear vein, the rabbits were sacrificed and the solid tumor mass of the periphery of the tumors was resected. After fixation in 70% ethanol and embedding in paraffin, tumor specimens of 4-μm thick sections were obtained and the BrdU-positive cells were stained with anti-BrdU MoAb (Becton Dickinson) by indirect immunoperoxydase staining (Vectastain ABC Kit, Vector Laboratories). The percentage of stained cells in relation to the total 300～500 tumor cells in the microscopic fields was defined as the staining index. Histological observation with hematoxylin and eosin staining was also performed in the specimen as part of this examination.

Finally, anti-BrdU MoAb staining was observed in 1 of the tumors 21 days after 20 Gy irradiation which had a co-existing defect and positive site of ^{201}TlCl uptake within the tumor, and the difference between these sites was compared (Fig. 9).

Statistics

The means and standard errors of the means were calculated for each group, and compared by Wilcoxon's rank sum test. P-values less than 0.05 were considered significant.

Results

All the 41 various-sized tumors at 7 days after implantation exhibited a positive uptake of ^{201}TlCl and even the smallest tumor ($10\times15\times20$ mm) showed an intensive accumulation. The mean tumor uptake ratio of these 41 non-irradiated tumors in this time was 2.79 ± 0.77. Relatively small tumors had no reduced uptake areas in the central portions at this time ; however, the follow-up scintigraphy revealed the tendency of a peripheral accumulation of ^{201}TlCl with a central area of reduced or defective uptake as the tumor grew (Fig. 1 (A)).

Comparison of the early (1 min after the injection) and delayed (60 min) static images in 11 tumors containing both non-irradiated and irradiated tumors revealed no alteration in the distribu-

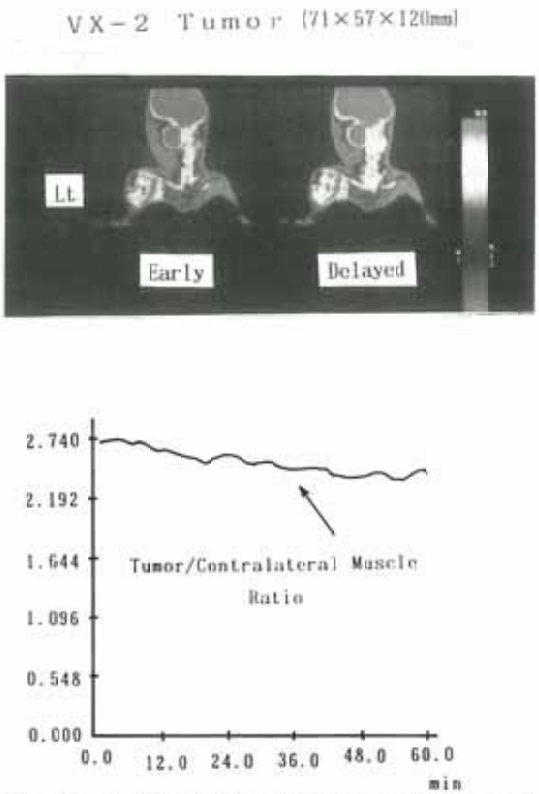

Fig. 1 (A) Early and delayed images of ^{201}TlCl scintigraphy in a VX-2 tumor with a size of 71×57×120 mm (top)
A peripheral accumulation of ^{201}TlCl with a central area of reduced uptake was seen. Comparison of the images showed no alteration in the distribution of ^{201}TlCl between the early and delayed ones.
Changes of tumor uptake ratio of ^{201}TlCl with time (bottom).
The highest value of the ratio was observed immediately after the injection of ^{201}TlCl, followed by a gradual decrease.

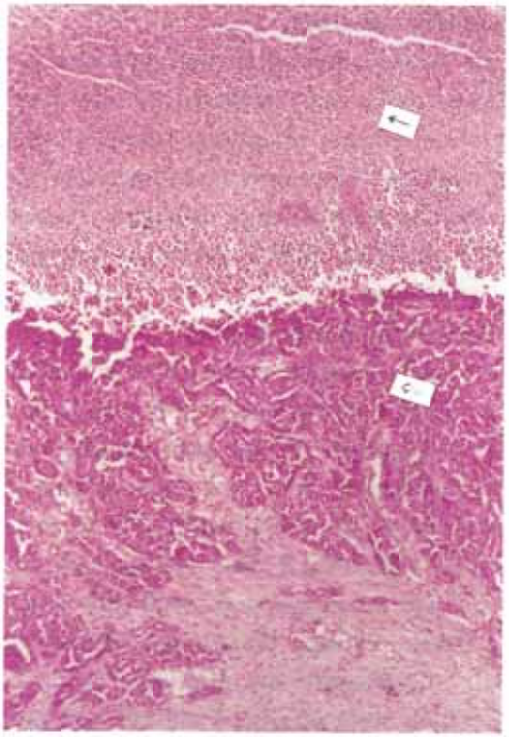

Fig. 1 (B) Histological specimen of the tumor in Fig. 1 (A) showing that the central necrosis (→) and viable tumor cells were seen only in the peripheral parts of the tumor (⇨) (H & E stain×100).

tion of ^{201}TlCl between the two images. All the tumors showed the highest value of tumor uptake ratio immediately after the ^{201}TlCl injection, followed by a gradual decrease (Fig. 1 (A)). In view of these results, the tumor uptake ratio obtained from the summation of the data during the first 5-10 minutes, which was relatively soon after the ^{201}TlCl injection, was assessed in the present study.

The relationships between the tumor volumes and tumor uptake ratios of ^{201}TlCl in the various size, non-irradiated 41 tumors were investigated by 83 examinations. The results showed nearly constant values of tumor uptake ratio independently of the tumor size among these tumors (Pearson's correlation test : $r=-0.1437$, $t=1.307$ N. S. and the mean tumor uptake ratio was 2.71 among the tumors studied), and revealed that neither significant increase nor decrease of the values of tumor uptake ratios occur with tumor growth (Fig. 2).

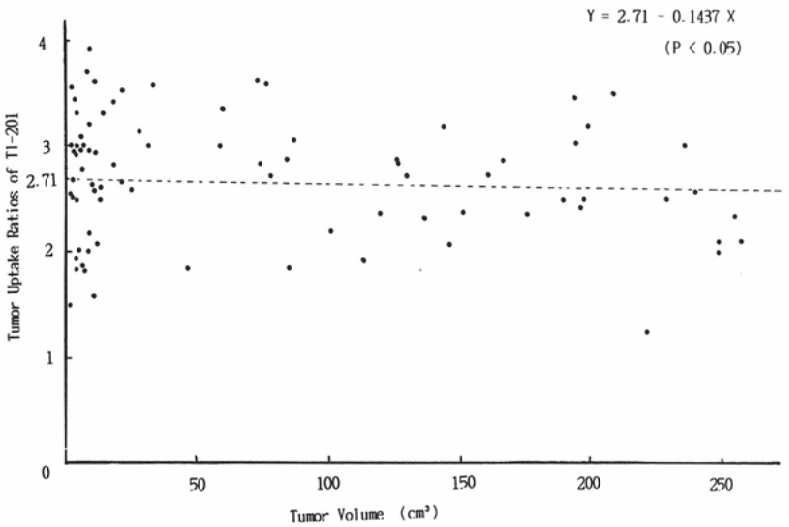

Fig. 2 The relationships between tumor volumes and tumor uptake ratios of ^{201}TlCl in the non-irradiated VX-2 tumors (83 examinations). An constant value (2.71) of tumor uptake ratio independently of various tumor volume was obtained (Pearson's correlation test: $Y=2.71-0.1437\,X$, $t=1.307$, $P<0.05$).

Dose-response curves for tumor growth are shown in Fig. 3. Each curve gives the mean and standard deviation of the data. The 8 control tumors showed a rapid tumor growth. The 9 tumors which were 20 Gy-irradiated showed no tumor shrinkage as a whole, despite of the irradiation, while some of the tumors showed progressive tumor shrinkage as shown in Fig. 5. Regrowth in these tumors began 28 days after irradiation. In contrast, 40 Gy-irradiated tumors showed progressive tumor shrinkage until the lesion disappeared completely between 28 and 35 days after irradiation.

Changes of tumor uptake ratios of ^{201}TlCl in the time course after irradiation are shown in Fig. 4. The tumor uptake ratios measured after treatment were normalized compared to the ratios at the time of treatment (1.0) in terms of relative tumor uptake ratio. When comparing the tumor growth curve (Fig. 3) and alteration of tumor uptake ratio, the control group showed nearly constant uptake ratios despite the rapid tumor growth, indicating that the uptake ratio measured in the highest activity site within the tumor was independent of tumor volume. The 20 Gy-irradiated group, as a whole, also showed no alteration of tumor uptake ratios at 7 days after irradiation. Subsequently, the values dramatically decreased at 14 days, while the tumor volumes were mostly the same as those on 7 days after irradiation. Thereafter, the values again increased at 21 days, before the tumor regrowth was observed at 28 days. In the 40 Gy-irradiated group, the relative tumor uptake ratios of 0.76 ± 0.32 were significantly lower than those of control tumors which were 0.96 ± 0.20 ($P<0.05$), and the ratios continued to decrease with progressive tumor shrinkage or their disappearance.

The 20 Gy-irradiated tumors were divided into two subgroups according to the values of tumor uptake ratios at 7 days after irradiation: in subgroup A, the values decreased more than 30% compared to pre-irradiation and in subgroup B, the values either decreased by only less than 30% or increased when compared to pre-irradiation. This allowed the observation of different tumor growth patterns between these two subgroups. Both subgroup A and B showed a slight tumor growth at 7

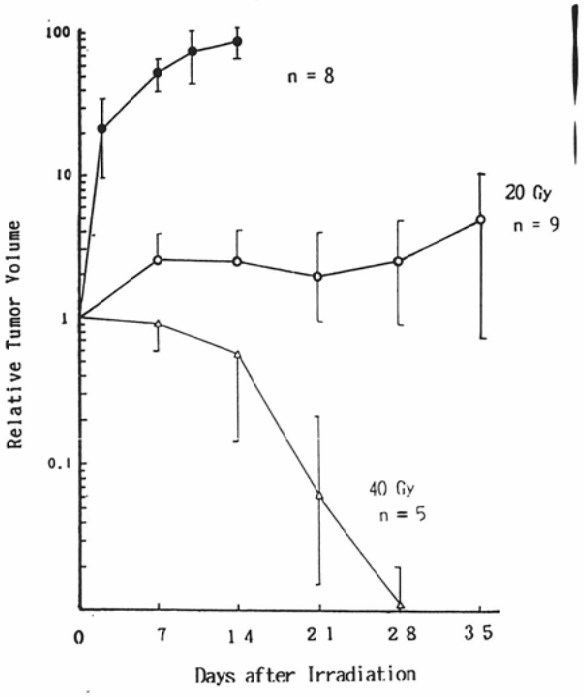

Fig. 3 Tumor dose-response growth curves after irradiation: Tumor volumes measured after irradiation were normalized at the time of treatment (1.0).
Each curve gives the mean and standard deviation of the data.

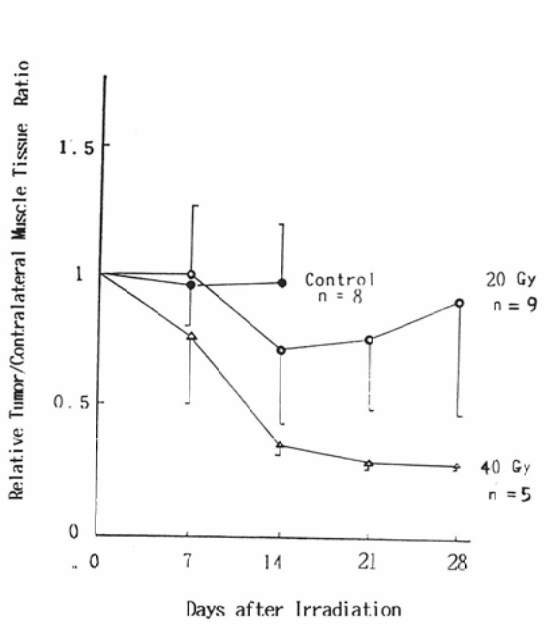

Fig. 4 Changes of tumor/contralateral muscle tissue ratio of ^{201}TlCl activity after irradiation: The tumor uptake ratios measured after treatment were normalized compared to the ratios at the time of treatment (1.0).
Each curve gives the mean and standard deviation of the data.

days post-irradiation, however, subgroup B showed gradual growth and subgroup A showed progresive tumor shrinkage thereafter (Fig. 5). Before the irradiation, there were no significant differencies between these subgroups in both of the mean tumor volume and tumor uptake ratio (N. S.) (the mean tumor volume and uptake ratio of subgroup A were $9.34\pm2.59\times10^3$mm^3 and 2.75 ± 0.64, and those of subgroup B were $8.67\pm2.26\times10^3$mm^3 and 2.83 ± 0.74, respectively.)

With regard to the time-course changes of tumor uptake ratios of these subgroups, the ratios of subgroup B had higher values on Day 7 after irradiation than those of pre-irradiation and thereafter showed reduced values once on Day 14 followed by further increase. Contrarily, the values of subgroup A showed constant lower values than those of pre-irradiation, and continued to decrease with time. The mean value of the relative tumor uptake ratio of subgroup A on Day 7 was reduced despite the increased tumor volume compared to pre-irradiation, and the value was significantly lower than that of subgroup B (0.61 ± 0.09 and 1.27 ± 0.74, respectively, $P<0.05$). (Fig. 6).

Two representative tumors of subgroups A and B are shown in Fig. 7. The top tumor with a size of $29\times22\times30$ mm, one of the subgroup A tumors, had 3.13 of tumor uptake ratio before 20 Gy-irradiation. The uptake ratio (1.97) decreased 37.1% on Day 7 compared to pre-irradiation, despite of slightly increase of tumor size. ^{201}TlCl scintigraphy performed 4 weeks later showed no

Comparison of tumor growth curves in the tumors which showed different response of ^{201}TlCl uptake on Day 7 after 20 Gy of irradiation

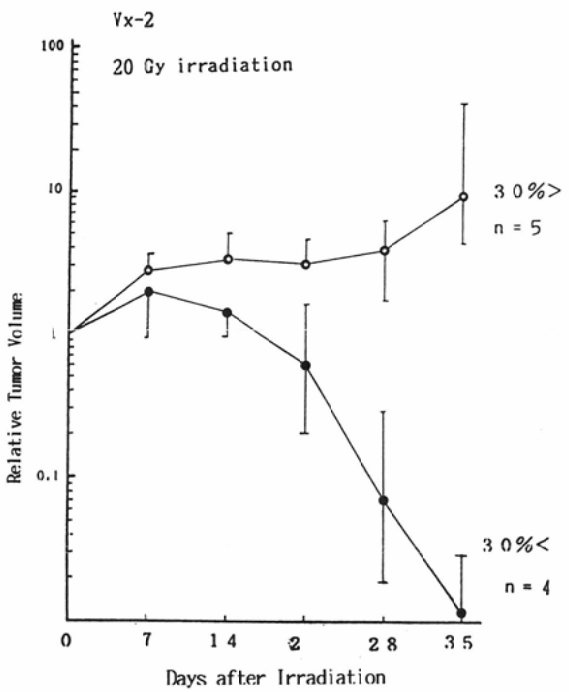

Fig. 5 Comparison of tumor growth curves in the tumors showing a different response of ^{201}TlCl uptake 1 week after 20 Gy-irradiation
Subgroup A : tumor uptake ratios decreased more than 30% compared to pre-irradiation on Day 7 after irradiation.
Subgroup B : The ratios decreased only less than 30% or increased compared to pre-irradiation at this time.

Changes of tumor/contralateral muscle tissue ratio of ^{201}TlCl activity after 20 Gy of irradiation

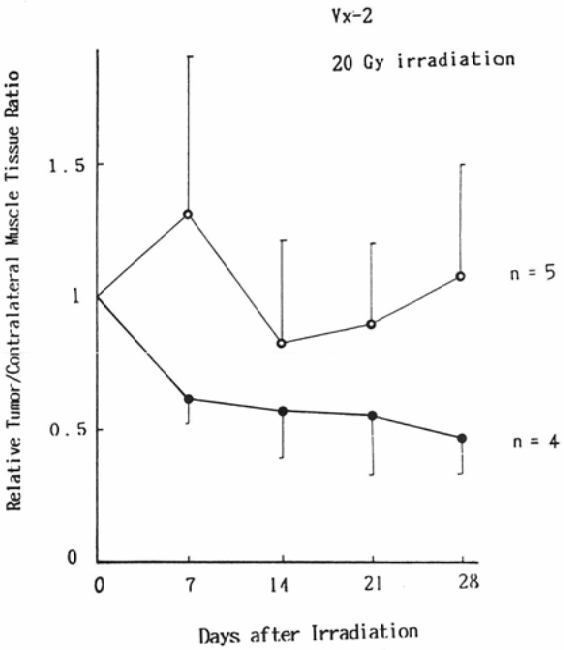

Fig. 6 Changes of tumor/contralateral muscle tissue ratio of ^{201}TlCl activity after 20 Gy-irradiation
The tumor uptake ratios measured after treatment were normalized compared to the ratios at the time of treatment (1.0).
Each curve gives the mean and standard deviation of the data.

abnormal accumulation in the tumor-bearing limbs and no tumor cells were found histologically. The bottom tumor, one of the subgroup B, which had nearly as same size and tumor uptake ratio as the top tumor before irradiation, however, it showed increse of the tumor uptake ratio (4.26) on Day 7 after 20 Gy-irradiation. This tumor grew 10.3 times larger than the pre-irradiation size 4 weeks after irradiation.

The histological investigation of the control tumors revealed that the relatively small, solid tumor with no ^{201}TlCl defect had only small regions of necrosis in the central portion. When peripheral accumulation of ^{201}TlCl was seen with a central defect, the tumor always had a large region of necrosis with fluid retention in the central portion, and a relatively thin layer of non-degenerating cells was seen in the peripheral portion (Fig. 1 (B)). Irradiated tumors also showed the same findings as seen in the control tumors ; however, the solid portion, especially in the 40 Gy-irradiated tumors, had many degenerating tumor cells compared to the control tumors, and this was unrelated to tumor size (Fig. 8). Cystic formation containing massive fluid was observed when a completely defective

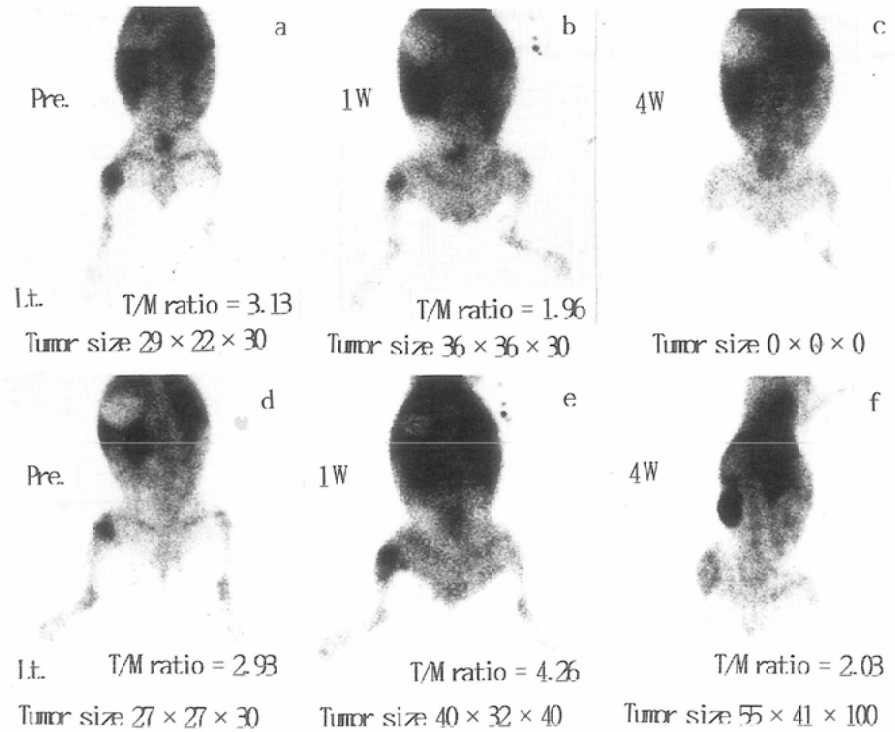

Fig. 7 (Top) : The other tumor, which disappeared 28 days after irradiation. The uptake ratio decreased 37.1% on Day 7 compared with the pre-irradiation values ((A), (B)). ^{201}TlCl scintigraphy performed 28 days showed no abnormal accumulation in the tumor-bearing limb. The histological survey revealed no tumor cells (C).
(Bottom) : The tumor, in which the tumor uptake ratio on Day 7 after irradiation increased 45% compared to the pre-irradiation values ((D), (E)), showed a tumor growth 10.3 times larger than the pre-irradiation size 21 days after irradiation. ^{201}TlCl scintigraphy at this time showed a peripheral ring-like appearance surrounding a central area of defect of ^{201}TlCl within the tumor (F).

site of ^{201}TlCl without peripheral accumulation was seen within the tumor. Survey of the three rabbit thighs, which showed disappearance of ^{201}TlCl accumulation and palpable tumors 35 days after irradiation in the 20 Gy- and 40 Gy-irradiated tumors, revealed no tumor cells anywhere, only degenerated muscle tissue (Fig. 7).

The anti-BrdU MoAb stain index and tumor uptake ratios of ^{201}TlCl in 6 control tumors were 47.2 $\pm$7.03% and 2.96$\pm$0.29, respectively. In contrast, the anti-BrdU MoAb stain index in the tumors which received 40 Gy-irradiation 7 days previously was 2.9$\pm$0.21%, showing very poor staining. The tumor uptake ratios of ^{201}TlCl in the these irradiated tumors were 1.85$\pm$0.51, significantly lower when compared with those of the control tumors ($P<0.01$) (Fig. 8).

Finally, the tumor in which there was a co-existing high ^{201}TlCl uptake site (site A ; tumor uptake ratio of 2.23) and a defective uptake site (site B ; tumor uptake ratio of 0.76) is shown in Fig. 9. The histological specimen of site A had a large region of necrosis with a small area of degenerating cells, without staining of the cells by anti-BrdU MoAb. Contrarily, in site B, there were many viable tumor cells and many tumor cells stained by anti-BrdU MoAb.

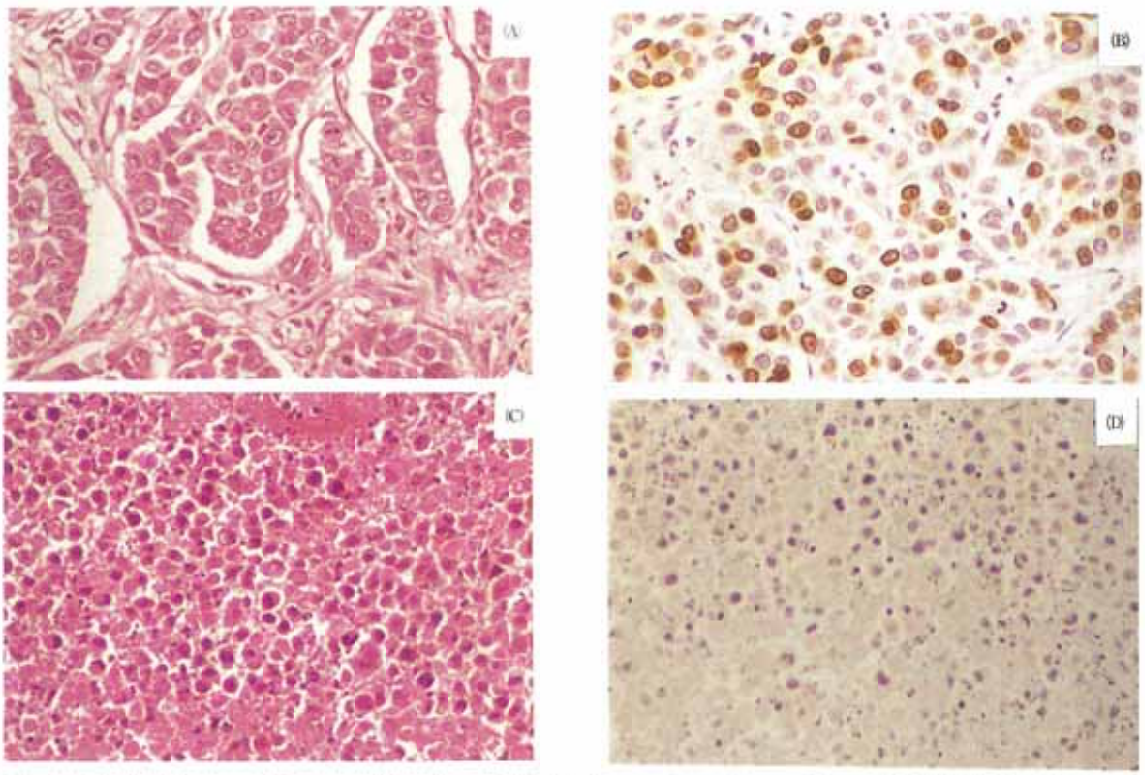

Fig. 8 (Top) : The control tumor with a size of 50×35×40 mm showed a positive anti-BrdU MoAb staining of 53.5% ((A): H&E stain, (B): BrdU stain). The tumor uptake ratio of ^{201}TlCl in this tumor was 3.03.
(Bottom) : The 40 Gy-irradiated tumors with a size of 54×38×43 mm showed many degenerative tumor cells in the peripheral parts of the tumor ((C): H&E stain). No tumor cells staining positive to anti-BrdU MoAb was found in this microscopyic field ((D): BrdU stain). This tumor uptake ratio of ^{201}TlCl was 1.44.

Discussion

^{201}Tl has an affinity for K^+ because it is the metal that belongs to III A of the periodic table and has an in vivo distribution similar to the K belonging to IA[8),9),11)]. Therefore, ^{201}Tl also has an affinity for the K^+ activating site of (Na^+-K^+) ATPase, which has a function in potasium metabolism as a supplier of the energy necessary for maintaining an asymmetric distribution of Na^+ and K^+ across the cell surface[8),13)]. Accelerated active transport of K^+ carried out by (Na^+-K^+) ATPase in the malignant tumor is said to be a trigger of ^{201}TlCl affinity for tumor cells[8),9)]. Ito et al[10)] also noted a high potassium content in the VX-2 tumors of rabbit and found a correlation between the uptake of ^{201}TlCl and that of ^{42}K in this tumor.

Moreover, some in vitro studies revealed that the level of (Na^+-K^+) ATPase was correlated with tumor growth potentiality[9)] and ^{201}TlCl uptake was related to the viability of the tumor cells[8)], and that ^{201}TlCl was hardly taken up by nonviable tumor cells[10)]. In addition, in an in vivo study, it was noted that ^{201}TlCl showed a high concentration in viable tumor tissue, less in connective tissue and hardly any accumulation in necrotic tumor tissue[4),10),12)].

Considering these findings, ^{201}TlCl scintigraphy is potentially useful for assessing the viability of tumor cells and for detecting residual tumor cells after radiation therapy. However, little has been

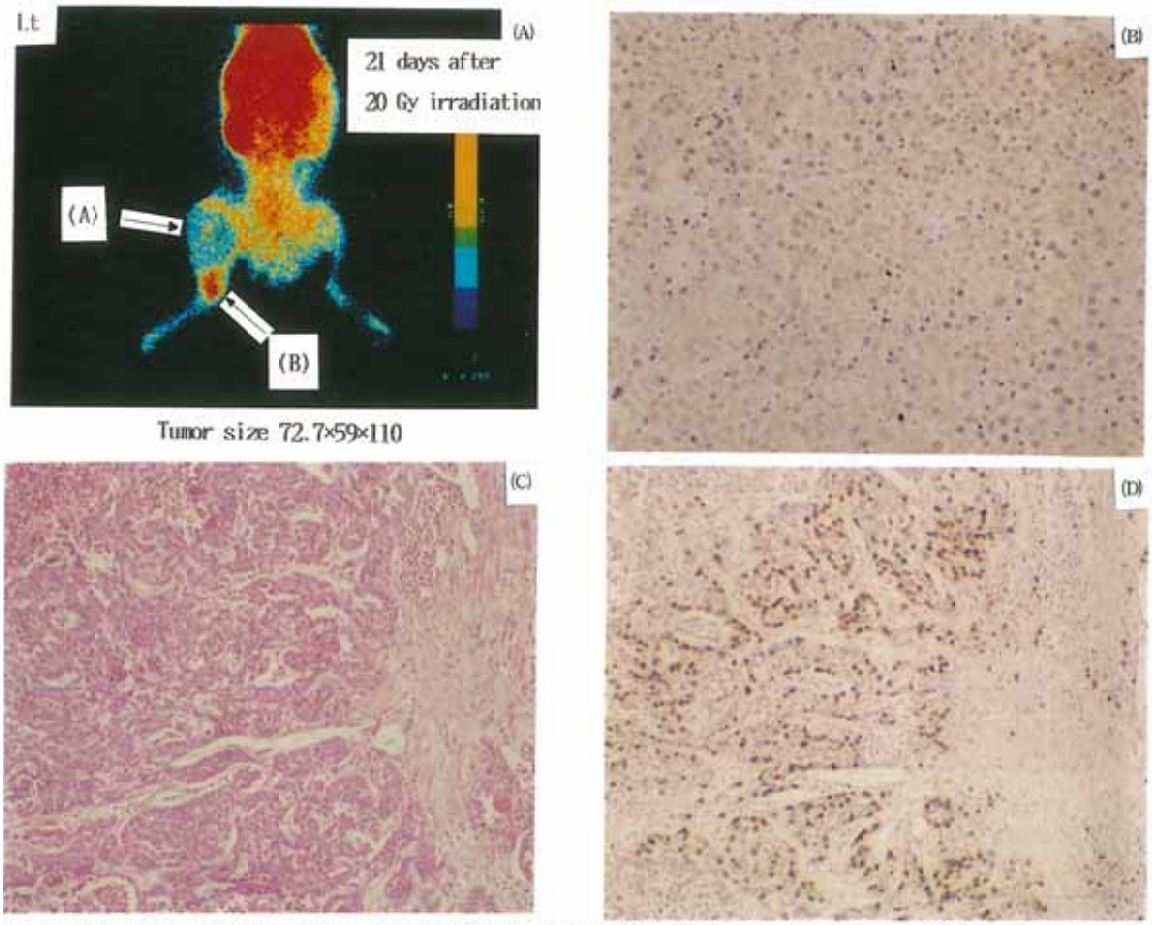

Fig. 9 The tumor in which both a high ^{201}TlCl uptake area (site A; tumor uptake ratio of 2.23) and a reduced uptake area (site B; tumor uptake ratio of 0.76) were co-existing at 21 days after 20 Gy of irradiation (A). The histological specimen of site A showed necrosis on the whole and no anti-BrdU MoAb staining (B). In contrast, that of site B showed many viable tumor cells stained by anti-BrdU MoAb ((C): H&E stain, (D): BrdU stain).

previously investigated in terms of the efficacy of ^{201}TlCl scintigraphy in monitoring the effects of radiation therapy.

In the present study, assessment of tumor uptake ratio of ^{201}TlCl was performed by measuring the intensive uptake area within the tumor, which existed mainly in the peripheral region in cases of relatively large tumors. As a result, the tumor uptake ratios showed no significant changes among the various size tumors (Fig. 2), and also showed nearly constant values of them with time in the rapidly growing control tumors (Fig. 4). The reasons of this unchanged tumor uptake independently of the tumor growth are considered as follows. In the medium sized tumors, reduced ^{201}TlCl activity in the necrotic tissue within the tumor suppresses increase of ^{201}TlCl activity due to growing viable tumor cells, although the reduced or defective area of ^{201}TlCl activity within the tumor cannot be seen on the scintigraphy. In fact, the present histological study showed frequent central necrotic degenerations even in the small tumors. And in the relatively large tumors, the non-reduced tumor uptake of ^{201}TlCl in the peripheral site reflects the constant tumor viabilty as same as the relatively

small tumors. This nearly constant value of the tumor uptake ratio independently of tumor volume is considered to be favorable for assessing the alteration of ^{201}TlCl uptake following irradiation in various size tumors.

A comparison of tumor growth curve and changes of tumor uptake of ^{201}TlCl after irradiation revealed a gradual and constant decrease with time after irradiation in the shrunken or resolved tumors after 20 or 40 Gy of irradiation. Tumor uptake of ^{201}TlCl on Day 7 after irradiation in these shrunken tumors was reduced compared to the rapidly growing control tumors. In the 20 Gy-irradiated tumors, different tumor growth patterns were observed between the subgroups divided according to the degree of tumor uptake of ^{201}TlCl on Day 7 after irradiation (Figs. 3-6). These results indicate that reduced ^{201}TlCl uptake following irradiation reflect the depressed proliferative activity induced by radiation therapy and also that evaluation of the altered ^{201}TlCl tumor uptake at a relatively early time following irradiation allows the prediction of subsequent growth.

Both of subgroups A and B of the 20 Gy-irradiated tumors showed slight tumor growth at 7 days after irradiation and had almost the same tumor volume; however, ^{201}TlCl uptake at this time was significantly different between these two subgroups (Figs. 5, 6, 7). Detection of these differences of ^{201}TlCl affinity, which is independent of tumor volume, is thought to be very important when monitoring the effects of radiotherapy. By the way, the ratios of subgroup B had higher values on Day 7 after irradiation than those of pre-irradiation. The precise reasons for this phenomenon are unknown, however, the early effect on the tumor vessels by irradiation, such as altered ^{201}TlCl permeability, can be considered. Kubota et al[1] also reported an unexpected increase of gallium-67 uptake by the tumors despite of suppressed tumor metaboilsm of glucose and amino acid, earlily following the irradiation. Further examinations are necessary to clarify whether the increse ^{201}TlCl uptake following irradiation occur exactly, scince the present data is obtained in the small numbers of animals.

The increased tumor uptake ratio of ^{201}TlCl seen prior to actual tumor regrowth in the 20 Gy-irradiated tumors is also an interesting finding (Fig. 3-6). Increased ^{201}TlCl uptake probably reflected the renewed increased tumor viability or proliferative potentiality before the actual growth. These results indicate that ^{201}TlCl scintigraphy can be used for prediction of tumor regrowth during follow-up study after irradiation.

Histological examination, especially as shown in the tumors with peripheral accumulation of ^{201}TlCl, revealed that ^{201}TlCl was hardly taken up by necrotic tissue within the tumor (Fig. 1(B)). Many degenerating tumor cells were observed in the solid portion of 40 Gy-irradiated tumors, accompanied with reduced ^{201}TlCl tumor uptake (Fig. 8). These histological findings suggest that ^{201}TlCl scintigraphy can reflect the viablity of tumor tissue.

Anti-BrdU MoAb was used to identify S-phase tumor cells and to evaluate their growth potentiality[14)-18)]. Staining by Anti-BrdU MoAb was remarkably poor in the tumors on Day 7 after 40 Gy-irradiation, even while the tumor size was larger than the control tumors with a stain index of 40-50%. This poor staining in the 40 Gy-irradiated tumors indicate the suppressed growth potentiality relatively soon after irradiation, and this was proven by the progressive tumor shrinkage as seen in the tumor growth curve (Fig. 3). The tumor uptake of ^{201}TlCl in these tumors was significantly lower than those of the control tumors. However, ^{201}TlCl uptake in these tumors seemed to be out

of proportion to the remarkably poor staining with Anti-BrdU MoAb. This is probably due to the discrepancy between (Na^+-K^+) ATPase activity and DNA synthesis activity in the irradiated tumor. These results showing that reduced ^{201}TlCl uptake is seen in the irradiated tumor with poor proliferative potentiality support the possibility that changes of ^{201}TlCl uptake relatively soon after irrradiation can reflect the changes of tumor growth potentiality.

Finally, ^{201}TlCl scintigraphy shown in Fig. 9 was interesting because it depicted two different uptake sites within the same tumor ; the high ^{201}TlCl uptake site was demonstrated to have viable and proliferative tumor cells confirmed by histological and Anti-BrdU MoAb staining examinations, while the defective site had a large region of necrosis. Specifically, ^{201}TlCl scintigraphy could depict the residual proliferative tumor site after radiation. Detection of these residual tumor sites after radiation is of great clinical importance when monitoring the therapeutic effects.

Conclusions

Feasibility of ^{201}TlCl scintigraphy for monitoring radiotherapeutic effects was evaluated by means of choice of only two levels of irradiation. Therefore, the present study may said a preliminary one, however, the results of ^{201}TlCl scintigraphy and simultaneously obtained findings of tumor growth curves, histological examination and anti-BrdU MoAb staining indicate that the follow-up study of ^{201}TlCl scintigraphy after irradiation may provide early prediction of tumor growth and delineation of residual viable, proliferative tumor tissue, in addition to contributing to estimation of the efficacy of therapy, independently to the tumor volumes.

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References

1) Kubota K, Ishiwata K, Kubota R, Yamada S, Tada M, Sato T : Tracer feasibility for monitoring tumor radiotherapy ; A quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorodeoxyuridine, L-[methyl-^{14}C] methionine, [6-^{3}H] thymidine, and gallium-67. J Nucl Med 1991 ; 32 : 2118-2123.
2) Okazumi S, Isono K, Enomoto K, Kikuchi T, Ozaki M, Yamamoto H : Evaluation of liver tumors using fluorine-18-fluorodeoxyglucose PET : Characterization of tumor and assessment of effect of treatment. J Nucl Med 1992 ; 33 : 333-339.
3) Daemen BJG, Elsinga PH, Mooibroek J, Paans AMJ, Wieringa AR, Konings AWT : PET measurement of hyperthermia-induced suppression of protein synthesis in tumors in relation to effects on tumor growth. J Nucl Med 1991 ; 32 : 1587-1592.
4) Black KL, Hawkins RA, Kim KT, Becker DP, Lerner C, Marciano D : Use of thallium-201 SPECT to quantitate malignancy grade of gliomas. J Neurosurg 1989 ; 71 : 342-346.
5) Tonami N, Shuke N, Yokoyama K, Seki H, Takayama T, Kinuya S, Nakajima K, Aburano T, Hisada K, Watanabe Y : Thallium-201 single photon emission computed tomography in the evaluation of suspected lung cancer. J Nucl Med 1989 ; 30 : 997-1004.
6) Sehweil AM, Mckillop JH, Milroy R, Sayed MA, Ziada G, Banham SW, Davidson KG, Ragib A, Omar YT, Abdel-dayem HM : ^{201}Tl scintigraphy in the staging of lung cancer, breast cancer and lymphoma. Nucl Med

Comm 1990 ; 11 : 263-269.
7) Matsuno S, Tanabe M, Kawasaki Y, Satoh K, Urrutia AE, Ohkawa M, Maeda M : Effectiveness of planar image and single photon emission tomography of thallium-201 compared with gallium-67 in patients with primary lung cancer. Eur J Nucl Med 1992 ; 19 : 86-95.
8) Muranaka A : Accumulation of radioisotopes with tumor affinity. II Comparison of the tumor accumulation of ^{67}Ga-citrate and ^{201}Tl-chloride in vivo. Acta Med Okayama 1981 ; 35 : 85-101.
9) Elligsen JD, Thompson JE, Frey HE, Kruuv J : Correlation of (Na^+-K^+) ATPase activity with growth of normal and transformed cells. Exp Cell Res 1974 ; 87 : 233-240.
10) Ito Y, Muranaka A, Harada T, Matsudo A, Yokobayashi T, Terashima H : Experimental study on tumor affinity of ^{201}Tl-chloride. Eur J Nucl Med 1978 ; 3 : 81-86.
11) Gehring PJ, Hammond PB : The interrelationship between thallium and potassium in animals. J Pharmacol Exp Ther 1967 ; 155 : 187-201.
12) Ando A, Ando I, Katayama M, Sanada S, Hiraki T, Mori H : Biodistributions of ^{201}Tl in tumor bearing animals and inflammatory lesion induced animals. Eur J Nucl Med 1987 ; 12 : 567-572.
13) Epstein A : Zur Frage des Kalium-Gehaltes in Krebsgeschwülsten. Z. Krebsforschung 1932 ; 38 : 63-74.
14) Gratzner HG : Monoclonal antibody to 5-bromo and 5-iododexyuridine ; A new reagent for detection of DNA neplication. Science 1982 ; 218 : 474-475.
15) Sasaki K, Ogino T, Takahashi M : In vitro BrdU labeling of solid tumors and immunological determination of labeling index. J Histotechnol 1987 ; 10 : 47-49.
16) Nagashima T, Hoshino T, Cho KG : The proliferative potential of ependymomas measured by in situ bromodeoxyuridine labeling. Cancer 1988 ; 61 : 2433-2438.
17) Oriuchi N, Tamura M, Shibazaki T, Inoue T, Watanabe N, Tateno M, Tomiyoshi K, Hirano T, Igarashi H, Endo K, Sasaki Y : Evaluation of ^{201}Tl SPECT in patients with glioma ; A comparative study with histological diagnosis, clinical feature and proliferative activity. Jpn J Nucl Med 1991 ; 28 : 1263-1271.
18) Nakanishi M, Fukushima M, Arai K, Koike T : Study of the anti-cancer effects of hyperthermia with immuno-histochemical technique using anti-BrdU monoclonal antibody. Jpn J Hyperthermic Oncol 1991 ; 7 : 131-145.