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Increased uptake of ^{99m}Tc-HL91 in tumor cells exposed to X-ray radiation

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^{99m}Tc-HL91, a hypoxic marker, may be a predictor of tumor response to radiotherapy and an indicator of tumor oxygenation in the course of treatment. In this study, serial changes in ^{99m}Tc-HL91 uptake were observed in the normoxic condition in a human bladder cancer cell line exposed to a single dose or a fractionated dose of 10 Gy with an x-ray beam. The uptake per cell increased during cell growth retardation induced by the irradiation. This finding indicates that ^{99m}Tc-HL91 uptake is affected by injury to cells due to radiation; it may therefore be difficult to correctly assess the tissue oxygenation status during radiotherapy with ^{99m}Tc-HL91.

Key words: ^{99m}Tc-HL91, tumor cell, hypoxia, radiotherapy

INTRODUCTION

THE EXISTENCE of hypoxic cells in heterogeneously perfused tumor tissue may result in its poor response to radiotherapy and chemotherapy,[1,2] so that the recognition of a hypoxic fraction prior to treatment may provide information on the prediction of therapeutic outcome. Nitroimidazoles which are trapped in hypoxic cells after bioreduction of the nitro group have been investigated for this purpose.[1,2] ^{99m}Tc-HL91 is a new agent lacking a nitroimidazole group but shows signs of hypoxia selectivity.[3–9] Although *in vivo* accumulation of ^{99m}Tc-HL91 was shown in both experimental tumors[7,8] and tumors in patients,[9] its mechanism is not yet clear. It is suggested that the uptake process involves no active transport, and the retention mechanism is independent of its metabolism and different from that of nitroimidazoles.[10]

^{99m}Tc-HL91 may be a predictor of tumor response to radiotherapy and an indicator of tumor oxygenation in the course of treatment.[1,2] Tumor reoxygenation after treatment may also be assessed by this tracer.[2] In this study, serial changes in ^{99m}Tc-HL91 uptake in irradiated tumor cells were observed to investigate what ^{99m}Tc-HL91 uptake during and/or after radiotherapy would mean.

MATERIALS AND METHODS

A human bladder carcinoma cell line, T24, was grown in RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal calf serum at 37°C under a 95% O_2 and 5% CO_2 atmosphere. Cells were harvested with 0.1% trypsin, washed with fresh medium and inoculated into 24 multiwell plates (Falcon 3047, Becton Dickinson and Company, Lincoln Park, NJ) at 3×10^4 cells per well in 1 m*l* of medium. On consecutive days, the number of cells was determined by trypan blue exclusion (n = 3). One day after the inoculation (day 0), the cells were exposed to a single dose of 10 Gy or a fractionated dose of 10 Gy (2 Gy for 5 days) at 4 Gy/min by means of an X-ray beam (150 keV, 15 mA) (MBR-1520R, Hitachi, Tokyo, Japan).

^{99m}Tc-HL91 (Nycomed Amersham, Buckinghamshire, England) was prepared with 2 m*l* of ^{99m}Tc-pertechnetate

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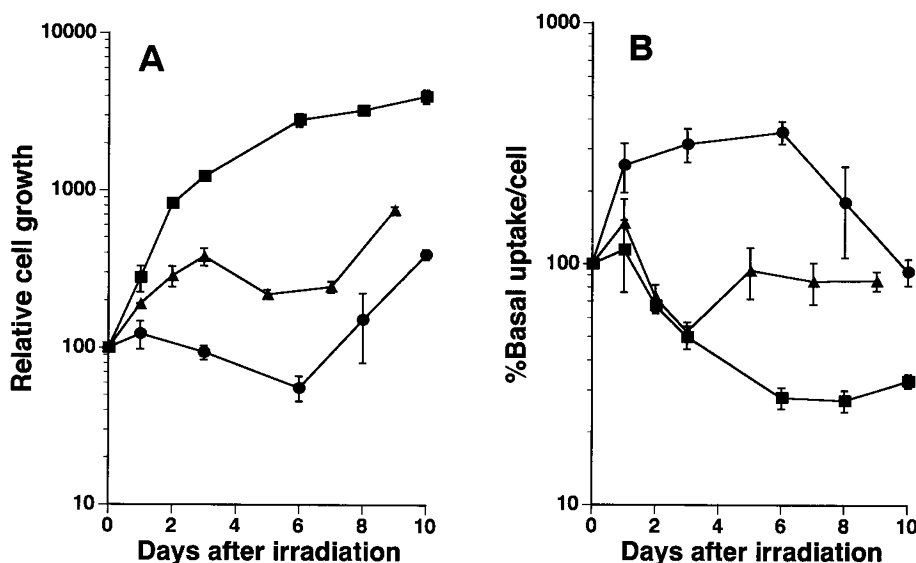

Fig. 1 Cell growth curves of T24 human bladder cancer cells (A) and uptake per cell of ^{99m}Tc-HL91 (B). Expressed as a percentage of values on day 0. ■, control cells; ▲, cells exposed to a fractionated 10 Gy (2 Gy for 5 days); ●, cells exposed to a single dose of 10 Gy.

(370 MBq/m*l*). On day 0 through day 10 after the irradiation, the cells were incubated with 370 KBq of ^{99m}Tc-HL91 in 200 μl of medium for 60 min in room air. The reaction was stopped by adding 1 m*l* of chilled phosphate-buffered saline (PBS), and the cells were then washed twice with 1 m*l* of PBS. Non-specific background activity was negligible after this washing procedure. The cells were harvested with 1 m*l* of 0.1% trypsin and the radioactivity was counted (n = 3). The cell-binding fraction of applied radioactivity per cell was calculated, and tracer uptake was expressed as a percentage of uptake on day 0. The assay was performed in the normoxic condition because it is not possible to keep the cells in the hypoxic condition for a long period. This observation therefore provides information on the uptake process in the irradiated cells only, not on the retention mechanism in the hypoxic condition.

RESULTS

Growth curves of the cells are shown in Figure 1A. Non-irradiated control cells showed signs of logarithmic growth during the observation period. On the other hand, cell growth delay was induced by an exposure to 10 Gy. A single 10 Gy dose inhibited cell growth more than a fractionated dose.

In the control cells, uptake of ^{99m}Tc-HL91 per cell decreased during a logarithmic cell growth. Uptake in the cells irradiated with a single 10 Gy dose increased while cell growth retardation was observed (Fig. 1B). In the cells exposed to a fractionated dose of 10 Gy, the uptake decreased during the initial increase in the number of cells up to day 3 and increased during the regression of the viable number of cells.

DISCUSSION

Reports on *in vivo* assessment of ^{99m}Tc-HL91 showed that the radioactivity in tumors reached peak at a few hours after the injection and was retained longer than that in normal tissues.[8,9] The slower kinetics of ^{99m}Tc-HL91 in tumors than in normal tissues suggests that the retention mechanism would likely determine the characteristics of its behavior in hypoxic tissues. Because the cellular uptake of ^{99m}Tc-HL91 was observed in the normoxic condition in this study, the results obtained may not precisely reflect its tracer kinetics. But, because process of uptake of ^{99m}Tc-HL91 involves no active transport,[10] the uptake (not the retention) must be independent of the oxygenation status of the cells, so that the results shown in this study can be applied to hypoxic cells.

The increased uptake of ^{99m}Tc-HL91 in the irradiated tumor cells is essentially similar to those previously reported with ^{201}Tl-chloride, ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin in the same cell line.[11] It is also known that ^{18}F-FDG uptake in cells increases after the exposure to radiation.[12] These findings indicate that the increase in tracer uptake in irradiated cells is a universal phenomenon which is independent of the inherent uptake mechanism of each tracer. Although the precise mechanism of this phenomenon is not clear, radiation-induced damage to structural integrity of the cell membrane and/or inactivation of transport systems are possible factors.[13]

Reoxygenation of tumor tissue would occur after radiation exposure, which enhances the therapeutic efficacy and may determine the final outcome of treatment; therefore assessment of the tissue oxygenation status during the therapeutic course would be of value in a clinical setting. Although reoxygenation of irradiated tumors has

been demonstrated in animal models, there is no direct evidence of this phenomenon in human tumors. Because the direct measurement of tissue oxygenation in patients is not feasible in most cases, scintigraphy with hypoxia-specific tracers surely plays a role in this assessment; but, as demonstrated in this study, the uptake of ^{99m}Tc-HL91 in the viable damaged cells increases, and this would make the scintigraphic assessment of tissue oxygenation difficult.

It is possible that the observation of its retention process, which would be a major factor in its cellular kinetics, may provide information for the better understanding of the kinetics of ^{99m}Tc-HL91 in irradiated cells; but we expect that washout of ^{99m}Tc-HL91 from the damaged cells may be enhanced similarly to those of ^{201}Tl, ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin as a result of a phenomenon non-specifically occurring after the radiation exposure.[11] Nevertheless, because tracer kinetics in tissues are not necessarily anticipated by its *in vitro* behavior, *in vivo* study with a xenograft model is required to evaluate the clinical value of ^{99m}Tc-HL91 during radiotherapy.

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