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# Evaluation of In-111 DTPA-paclitaxel scintigraphy to predict response on murine tumors to paclitaxel

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Our goal was to determine whether scintigraphy with $^{111}$In-DTPA-paclitaxel could predict the response to chemotherapy with paclitaxel. *Methods:* Ovarian carcinoma (OCA 1), mammary carcinoma (MCA-4), fibrosarcoma (FSA) and squamous cell carcinoma (SCC VII) were inoculated into the thighs of female C3Hf/Kam mice. Mice bearing 8 mm tumors were treated with paclitaxel (40 mg/kg). The growth delay, which was defined as the time in days for tumors in the treated groups to grow from 8 to 12 mm in diameter minus the time in days for tumors in the untreated control group to reach the same size, was measured to determine the effect of paclitaxel on the tumors. Sequential scintigraphy in mice bearing 10 to 14 mm tumors was conducted at 5, 30, 60, 120, 240 min and 24 hrs postinjection of $^{111}$In-DTPA-paclitaxel (3.7MBq) or $^{111}$In-DTPA as a control tracer. The tumor uptakes (% injection dose/pixel) were determined. *Results:* The growth delay of OCA 1, MCA-4, FSA and SCC VII tumors was 13.6, 4.0, –0.02 and –0.28 days, respectively. In other words, OCa 1 and MCA-4 were paclitaxel-sensitive tumors, whereas FSA and SCC VII were paclitaxel-resistant tumors. The tumor uptakes at 24 hrs postinjection of In-111 DTPA paclitaxel of OCA 1, MCA-4, FSA and SCC VII were $1.0 \times 10^{-3}$, $1.6 \times 10^{-3}$, $2.2 \times 10^{-3}$ and $9.0 \times 10^{-3}$ % injection dose/pixel, respectively. There was no correlation between the response to chemotherapy with paclitaxel and the tumor uptakes of $^{111}$In-DTPA-paclitaxel. *Conclusions:* Scintigraphy with $^{111}$In-DTPA-paclitaxel could not predict the response to paclitaxel chemotherapy. Although there was significant accumulation of the paclitaxel in the tumor cells, additional mechanisms must be operative for the agent to be effective against the neoplasm. $^{111}$In-DTPA-paclitaxel activity is apparently different from that of paclitaxel with Cremophor.

**Key words:** In-111 DTPA-paclitaxel, paclitaxel, chemotherapy

## INTRODUCTION

IT HAS LONG BEEN THE HOPE that scintigraphy would be useful for therapeutic drug monitoring (TDM), which is now conducting by measuring the concentration in blood[1] but the concentration of an anti-cancer agent does not predict the effect on tumor cells. Scintigraphy with radiopharmaceuticals demonstrates the *in vivo* biodistribution of an anti-cancer drug as well as the characteristics of drug resistance.[2] Can we expect a good response if a radiolabeled cytotoxic agent shows sings of high tumor uptake on scintigraphy?

Paclitaxel exerts its cytotoxic effects through its interference with microtubule assembly,[3,4] and is active against a broad range of cancers that are considered to be refractory to conventional chemotherapy.[5,6] If radiolabeled paclitaxel could predict the response to paclitaxel and select those patients to be treated, much expense and time would be saved.

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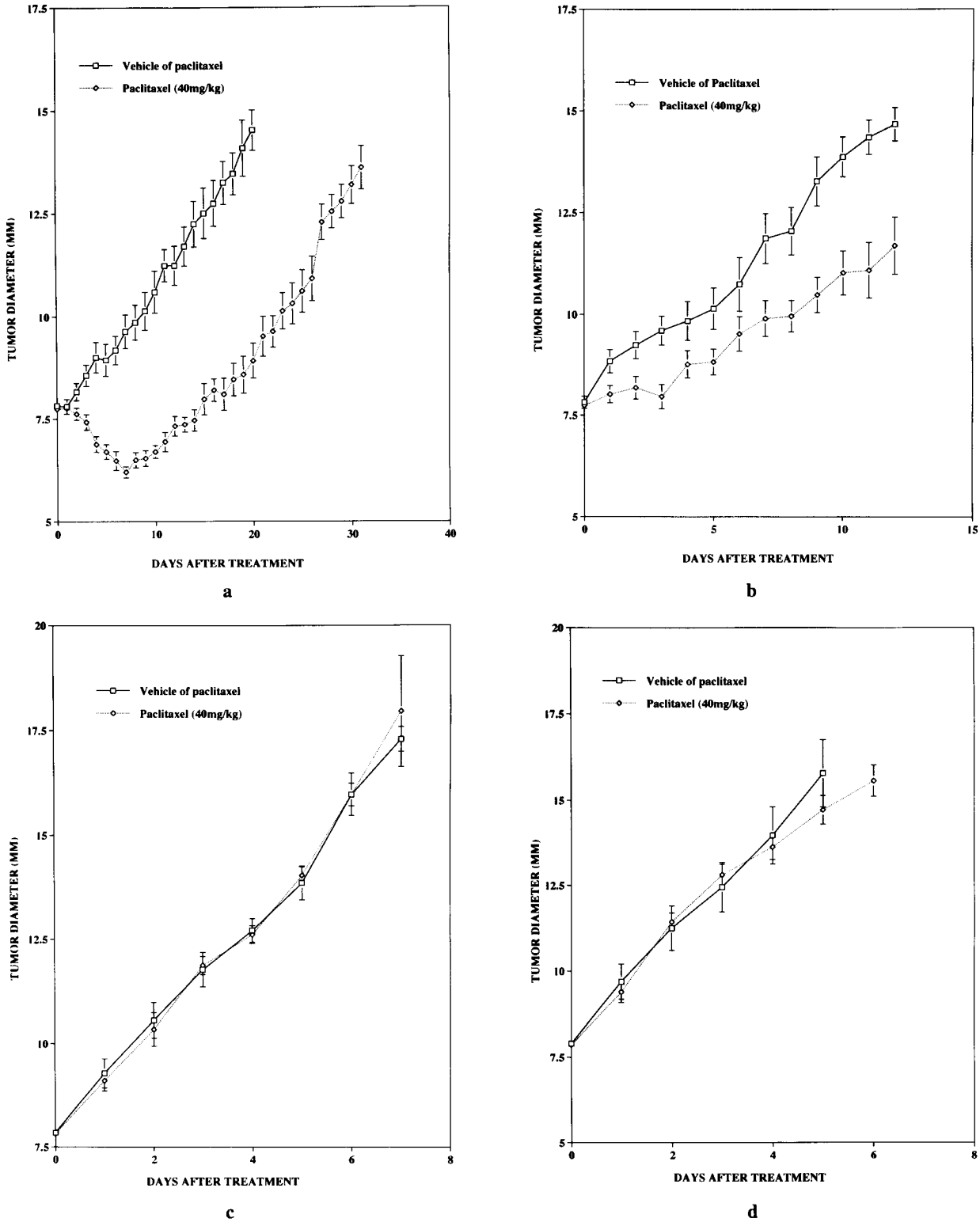
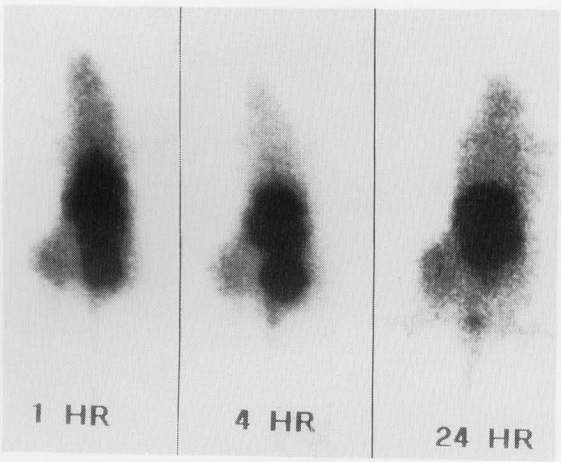
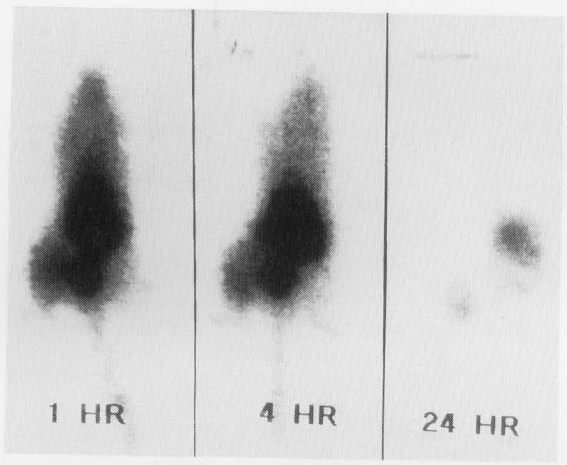



**Fig. 1** The growth curves in mice treated and untreated with paclitaxel (40 mg/kg). OCA 1 (a) and MCA-4 (b) tumors responded to paclitaxel but FSA (c) and SCC VII (d) tumors did not have sensitivity to paclitaxel.




a

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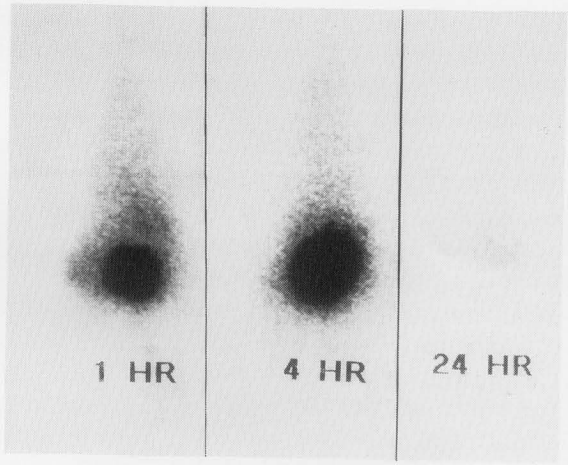


c

**Fig. 2** The scintigrams with $^{111}$In-DTPA-paclitaxel in tumor bearing mice; MCA-4 (a), FSA (b) and with $^{111}$In-DTPA in mice with MCA-4 tumor (c). The images with $^{111}$In-DTPA-paclitaxel revealed the liver uptake and excretion of $^{111}$In-DTPA-paclitaxel into the intestine through the hepatobiliary tract. In contrast to the images with $^{111}$In-DTPA that showed rapid clearance from the tumor (c), the retention of $^{111}$In-DTPA-paclitaxel in MCA-4 and FSA tumors were observed on the scintigrams with $^{111}$In-DTPA-paclitaxel (a, b).

We developed $^{111}$In-DTPA-paclitaxel at the University of Texas, M.D. Anderson Cancer Center[7] and present preliminary data in the evaluation of tumor uptake of the agent correlated with response to therapy in various tumor cell lines in mice.

## MATERIALS AND METHODS

C3Hf/Kam female mice (20–25 g), bred and maintained in a pathogen-free mouse colony in the Department of Experimental Radiotherapy of the University of Texas, M.D. Anderson Cancer Center, were used.[8] The mice were 3 months old at the beginning of the experiments and were housed 4–5 per cage. The animal models studied in this experiment were the fourth generation isotransplants of mammary carcinoma (MCA-4 tumors), the seventh generation isotransplants of ovarian carcinoma (OCA 1), the fifth generation isotransplants of fibrosarcoma (FSA) and squamous cell carcinoma (SCC VII).[8–10] Tumor cells were implanted in the muscle of the right thigh of mice by the inoculation of $5 \times 10^5$ viable tumor cells confirmed by trypan blue exclusion and phase microscopy.

Tumor growth assays were conducted by measuring three orthogonal tumor diameters with vernier calipers daily or every other day. When the tumors grew to 8 mm in average diameter, the mice were divided into two groups, (1) control and (2) treated with paclitaxel, of 5 mice each. A single dose of paclitaxel was given intravenously at a dose of 40 mg equiv. paclitaxel/kg body weight. Paclitaxel was first dissolved in absolute ethanol with an equal volume of cremophor. This stock solution (30 mg/m*l*) was further diluted (1 : 4 by volume) with sterile physiological solution within 10 min of injection. In the control groups, absolute alcohol/cremophor 1 : 1 diluted with saline (1 : 4) was used. After treatment, tumor growth was followed up until the average tumor size reached at least 12 mm in diameter.

The effect on tumor regrowth was expressed as the absolute growth delay, defined as the time in days for tumors treated with paclitaxel to grow from 8 to 12 mm minus the time in days for tumors in the control group to grow from 8 to 12 mm in diameter.

Into a solution of paclitaxel (100 mg, 0.117 mmol) in dry DMF (2.2 m*l*) was added 210 mg diethylenetriaminepentaacetic acid (DTPA anhydride, 0.585 mmol). The reaction mixture was stirred at 0°C overnight. The suspension was filtered to remove unreacted DTPA anhydride. The filtrate was poured into distilled water, stirred at room temperature for 20 min, and the precipitate of DTPA-paclitaxel was collected.

Into a 2 m*l* V-vial were added successively 40 $\mu l$ 0.6 M

sodium acetate (pH 5.3) buffer, 40 $\mu l$ 0.06 M sodium citrate buffer (pH 5.5), 20 $\mu l$ paclitaxel-DTPA solution in methanol (2% w/v) and 20 $\mu l$ $^{111}InCl_3$ solution (37 MBq) in sodium acetate buffer (pH 5.5). After an incubation period of 30 min at room temperature, the $^{111}In$ labeled paclitaxel-DTPA was collected in a methanol wash. After methanol was evaporated by passing through nitrogen gas, the labeled product was reconstituted in a suitable volume of saline.

HPLC was used to analyze the reaction mixture and the purity of $^{111}In$-DTPA paclitaxel. The system consisted of a LDC binary pump and a 100 mm × 8 mm (i.d.) Waters column filled with ODS 5 $\mu$m silica gel. The column was eluted at a flow rate of 1 m*l*/min with a gradient mixture of water and methanol (gradient from 0% to 85% methanol over 15 min). The gradient system was monitored with a NaI crystal detector and a Spectra-Physics UV/Vis detector.

When the tumors had grown to 12–14 mm in diameter, the mice each bearing a tumor (OCA 1, MCA-4, FSA and SCC VII tumor) were divided into two groups to assess the tumor uptakes of $^{111}In$-DTPA-paclitaxel or $^{111}In$-DTPA as a control tracer. The mice were anesthetized by i.p. injection of sodium pentobarbital, followed by i.v. injection of 3.7 MBq of either $^{111}In$-DTPA-paclitaxel or $^{111}In$-DTPA. Anterior planar images of the whole body were obtained at 5 min, 1, 2, 4 and 24 hrs postinjection of radiotracer with a single-head gamma camera equipped with a parallel-hole medium-energy collimator and connected to a computer. At all times, images were made with a 5-min preset time and stored in a 128 × 128 matrix. A symmetrical 20% energy window was used for both the 173 and 247 keV energy peaks.

Regions of interest (ROIs) were drawn over the tumor manually on the images obtained to measure the tumor uptakes of radiotracers and over the whole body on the image obtained at 5 min postinjection to define the total injection dose of radioactivity. The tumor uptake was defined as a percent of the injected dose per pixel (% ID/pixel). The results were expressed as the mean ± standard error of the mean (SEM).

The non-parametric Mann-Whitney U test was used to analyze data. A two-tailed p value less than 0.05 was considered to be a statistically significant difference.

## RESULTS

Purification with a Sep-Pak cartridge removed most of the $^{111}In$-DTPA which had a retention time of 2.7 min. A radiochromatogram of $^{111}In$-DTPA-paclitaxel correlated well with its corresponding UV chromatogram, indicating that the peak at 12.3 min was indeed the target compound. Paclitaxel had a retention time of 17.1 min under the same chromatographic conditions. The radiochemical purity of the final preparation was 90% and the radiochemical yield was 84%.

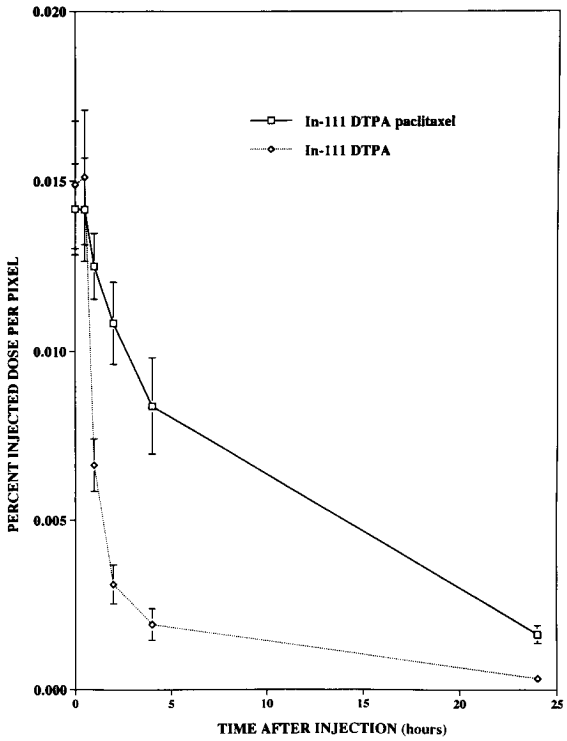


**Fig. 3** The time activity curves of $^{111}In$-DTPA-paclitaxel and $^{111}In$-DTPA in MCA-4 tumor. The tumor uptakes of $^{111}In$-DTPA-paclitaxel decreased along with time after the injection but the wash-out from tumors were slower than that of $^{111}In$-DTPA.

**Table 1** Results of tumor growth delay assay

| Tumor type | Treatment | Growth delay date | Statistics |
|---|---|---|---|
| MCA-4 | Vehicle of Paclitaxel | 8.80 ± 1.30 | |
| | Paclitaxel | 12.76 ± 1.29 | $p < 0.01$ |
| OCA 1 | Vehicle of Paclitaxel | 13.48 ± 1.55 | |
| | Paclitaxel | 27.12 ± 0.85 | $p < 0.001$ |
| FSA | Vehicle of Paclitaxel | 3.24 ± 0.24 | |
| | Paclitaxel | 3.22 ± 0.25 | N.S. |
| SCC VII | Vehicle of Paclitaxel | 2.70 ± 0.47 | |
| | Paclitaxel | 2.42 ± 0.22 | N.S. |

Each group included 5 mice. Growth delays were given as the duration when tumor regrowed from 8 to 12 mm in diameter. Data were expressed as mean and SEM. Statistics: Student's t-test or non parametric analysis (if SD is significant difference)

Paclitaxel exerted a significant antitumor effect on OCA 1 and MCA-4 but was ineffective against FSA and SCC VII tumors (Table 1). The absolute growth delay of OCA 1, MCA-4, FSA and SCC VII tumors was 13.6, 4.0, −0.02 and −0.28 days, respectively. Tumor regrowth curves are shown in Figure 1.

The scintigrams in tumor bearing mice revealed liver uptake and excretion of $^{111}In$-DTPA-paclitaxel into the intestines through the hepatobiliary tract (Fig. 2). Although the images with $^{111}In$-DTPA showed rapid clear-

**Table 2** Results of uptake of $^{111}$In-DTPA-paclitaxel and $^{111}$In-DTPA

| Tumor | | Times after injection | | | | |
|---|---|---|---|---|---|---|
| | | 0.5 hr | 1 hr | 2 hr | 4 hr | 24 hr |
| MCA-4 | Paclitaxel* | 14.2 ± 3.7† | 12.5 ± 2.4‡ | 10.8 ± 1.2‡ | 8.4 ± 1.4‡ | 1.6 ± 0.3‡ |
| | n | 6 | 6 | 6 | 6 | 6 |
| | DTPA§ | 15.1 ± 3.4 | 6.6 ± 0.7 | 3.1 ± 1.3 | 1.9 ± 0.5 | 0.3 ± 0.1 |
| | n | 3 | 5 | 5 | 5 | 3 |
| OCA 1 | Paclitaxel | 8.5 ± 0.6‡ | 6.7 ± 5.4 | 5.8 ± 0.4‡ | 4.3 ± 1.3‡ | 1.0 ± 0.2‡ |
| | n | 7 | 7 | 7 | 7 | 7 |
| | DTPA | 4.9 ± 1.2 | 4.5 ± 1.7 | 3.2 ± 0.7 | 1.2 ± 0.3 | 0.3 ± 0.1 |
| | n | 7 | 7 | 7 | 7 | 7 |
| FSA | Paclitaxel | 16.6 ± 1.3 | 15.3 ± 1.3‡ | 12.8 ± 0.1 | 9.9 ± 1.7 | 2.2 ± 0.3‡ |
| | n | 6 | 6 | 6 | 6 | 6 |
| | DTPA | 18.5 ± 2.5 | 10.7 ± 0.6 | not done | 4.6 ± 1.7 | 0.6 ± 0.0 |
| | n | 4 | 4 | | 4 | 4 |
| SCC VII | Paclitaxel | 16.4 ± 1.3 | 17.3 ± 1.4 | 18.4 ± 2.0 | 19.1 ± 2.8 | 9.0 ± 2.0‡ |
| | n | 7 | 7 | 7 | 5 | 3 |
| | DTPA | 21.1 ± 7.0 | 19.3 ± 6.5 | 18.2 ± 6.7 | 16.2 ± 6.0 | 1.2 ± 0.2 |
| | n | 5 | 5 | 5 | 4 | 3 |

* $^{111}$In-DTPA-paclitaxel, † % injection dose per pixel × $10^{-3}$; Data are given as mean ± standard error of mean
‡ significant difference from tumor uptake of $^{111}$In-DTPA ($p < 0.05$), § $^{111}$In-DTPA

ance from the tumor, the retention of $^{111}$In-DTPA paclitaxel was observed in tumors (Fig. 2).

The uptake of $^{111}$In-DTPA-paclitaxel in OCA 1, MCA-4, FSA and SCC VII tumors decreased with time after the injection but wash-out from the tumors was slower than that of $^{111}$In-DTPA (Table 2, Fig. 3). Uptakes at 24 hrs postinjection of $^{111}$In-DTPA-paclitaxel in OCA 1, MCA-4, FSA and SCC VII tumor were $1.0 \times 10^{-3}$, $1.6 \times 10^{-3}$, $2.2 \times 10^{-3}$ and $9.0 \times 10^{-3}$ % injection dose/pixel, respectively, which were significantly higher than those with $^{111}$In-DTPA as a control radiotracer (Table 2). Although OCA 1 was the tumor most sensitive to paclitaxel, tumor uptake in OCA 1 tumor at 1, 4 and 24 hrs postinjection of $^{111}$In-DTPA-paclitaxel was significantly lower than that in MCA-4, FSA and SCC VII tumor. There was no correlation between the response to chemotherapy with paclitaxel and tumor uptake of $^{111}$In-DTPA-paclitaxel (Tables 1 and 2).

## DISCUSSION

Assessment of the functional characteristics of the tumor is important in planning treatment. Anatomical information provided by newer imaging methods such as ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) is not adequate. The nuclear medicine imaging with SPECT and PET can be used to monitor tumor therapy,[11,12] to evaluate tumor metabolism, nucleic acid synthesis and drug uptake.[13] Various radiolabeled anticancer drugs have been developed in the hope of predicting the response to chemotherapy,[14–17] but the results and mechanisms predicting the response to chemotherapy with radiolabeled anticancer drugs were in conflict.

At first we expected that tumors with high uptake of $^{111}$In-DTPA-paclitaxel may respond to chemotherapy with paclitaxel because paclitaxel caused a dose-dependent decrease in the lag time for microtubule assembly.[4] This was also suggested by an *in vitro* study showing that the paclitaxel-resistant cells, J1.T1 cell line, which have a multidrug-resistance (MDR) phenotype, accumulated only 10% of $^{3}$H labeled paclitaxel found in the paclitaxel sensitive cells.[18,19] All 4 tumor cell lines used in our study showed specific uptakes of $^{111}$In-DTPA-paclitaxel, and in general there was no difference between paclitaxel sensitive OCA 1 and MCA-4 tumors and paclitaxel resistant SCC VII and FSA tumors in the uptake of $^{111}$In-DTPA-paclitaxel. The antineoplastic mechanism of paclitaxel mainly stabilizes tubulin polymerization resulting in the arrest of mitosis and apoptotic death of tumor cells. Since paclitaxel has a binding site on the microtubules, this may be the mechanism of $^{111}$In-DTPA-paclitaxel tumor uptake.[7] Although modification of the chemical structure of paclitaxel by labeling $^{111}$In and DTPA may cause a change in the mechanism of paclitaxel tumor uptake, we have confirmed that DTPA paclitaxel has antineoplastic action on OCA 1 and MCA-4 tumors like paclitaxel (unpublished data). Paclitaxel-resistant tumors (SCC VII tumor) also exhibited mitotic arrest after injection of paclitaxel although it did not have an antitumor action after mitotic arrest,[20] which is consistent with a significant uptake of $^{111}$In-DTPA-paclitaxel in SCC VII tumors. The accumulation of paclitaxel in the tumor cells is undoubtedly a prerequisite but not sufficient for successful treatment. Tumor uptake of $^{111}$In-DTPA-paclitaxel reflects the accumulation of paclitaxel by tumor cells, but the degree of

accumulation does not reflect the tumor cell sensitivity. It is quite possible that some cellular factors determine whether paclitaxel will be cytotoxic to tumor cells.

Although scintigraphy with $^{111}$In-DTPA-paclitaxel failed to predict tumor response to the chemotherapy with paclitaxel, it may be useful in predicting drug toxicity as does $^{123}$I-labeled digoxin.[21] In contrast to the lipophilic characteristics of paclitaxel, $^{111}$In-DTPA paclitaxel is water soluble and may therefore provide different biodistribution than paclitaxel. There was high liver uptake and hepatobiliary excretion, similar to paclitaxel metabolism and its excretion route. Although hepatic metabolism, biliary excretion and fecal elimination appear to be responsible for most of the systemic clearance, the optimal dose of paclitaxel for patients with liver dysfunction has not been determined.[3] Even if the same race of tumor bearing mice, C3Hf/Kam female mice, was employed in this study, there was a significant difference in liver uptake at 24 hr postinjection (Fig. 2a, b).[7] Scintigraphy may help to optimize chemotherapy for patients with hepatic dysfunction by monitoring the hepatobiliary clearance of $^{111}$In-DTPA-paclitaxel.

Further study to assess the mechanism of tumor uptake of $^{111}$In-DTPA-paclitaxel is needed to determine the usefulness of scintigraphy with this compound. It is speculated that there would be no therapeutic effect of paclitaxel if there is no significant uptake of $^{111}$In-DTPA-paclitaxel in tumors.

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