Evaluation of Carbon-14-Colchicine Biodistribution with Whole-body Quantitative Autoradiography in Colchicine-sensitive and -resistant Xenografts

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Quantitative autoradiography (QAR) with radiolabeled monoclonal antibodies in xenografted animals has been extensively described in the past, either on individual tissues or on the whole body. We applied whole-body QAR to identify multidrug resistant tumors using ^{14}C-colchicine (^{14}C-CHC). **Methods:** Two groups of five animals each were xenografted with CHC-sensitive and CHC-resistant human neuroblastoma cells. Animals were injected intravenously with 4 μCi/0.11 μmole ^{14}C-CHC per gram of body weight and sacrificed after 60 min. Whole-body QAR was carried out using 25-μm thick sections. **Results:** Fusion images allowed direct comparison of ^{14}C-CHC uptake in tumor and nontumor tissues. Mean ^{14}C-CHC distribution in sensitive and resistant tumors was 882.0 ± 43.6 and 399.6 ± 157.7 nCi/g corresponding to 24.5 ± 1.21 and 11.1 ± 4.38 nmole/g, respectively ($p < 0.001$), with normal tissue distribution in both groups being similar. Three-dimensional QAR showed that the uptake of ^{14}C-CHC was in the cellular zones of the tumor. This method has potential in biodistribution studies of novel radiopharmaceuticals such as ^{14}C-CHC. **Conclusion:** These studies further suggest that PET imaging of ^{11}C-CHC is feasible to distinguish between sensitive and resistant tumor deposits in vivo.

Key Words: multidrug resistance; colchicine; three-dimensional fusion imaging; biodistribution

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Several reports (*1–3*) have described whole-body quantitative autoradiography (QAR) with radiolabeled monoclonal antibodies (MAbs) in xenografted animal models; the method can detect preferential uptake of radioantibody in tumors as small as 0.1–0.2 mm in diameter. QAR along with H & E histological staining can register the penetration of the drug in various tumor and nontumor tissues.

Multidrug resistance (MDR) is thought to be a major obstacle in the effective treatment of cancer with natural chemotherapeutic agents (*4–9*). P-glycoprotein, an MDR gene product, is known to function as an ATP-dependent efflux pump which reduces intracellular drug accumulation in resistant tumor cells (*4–7*). Colchicine (CHC) (Fig. 1), a naturally occurring alkaloid and a potent inhibitor of cellular mitosis, is a member of MDR group of drugs and is relatively easy to label with ^{11}C, particularly at the chemoreactive vinyl ester site on the [C-ring, methoxy]-CHC.

A ^{11}C-labeled form of CHC, at a specific activity suitable for in vivo PET studies, has been successfully synthesized in our facility (Finn, RD, *personal communication*).

Since animal tumor models are sensitive and resistant to CHC, they have shown differential uptake (*10,11*). We applied whole-body autoradiography to identify multidrug resistant tumors using ^{14}C-CHC. A surface matching three-dimensional technique (*12*) was employed to coregister multiple H & E and QAR slices. Whole-body autoradiography allows exquisite matching of anatomical information with QAR. Anatomic structures with QAR permit accurate determination of spatial and organ distribution of radioactivity that is crucial to understanding biodistribution and dosimetry.

Identification of MDR in vivo could be an important aid in the design of effective chemotherapeutic regimen. Quantitation of p-glycoprotein in tumor biopsy specimen has been carried out by immunohistochemical (*13*) and electrophoretic (*14*) methods to identify the resistant cell population. A direct method for clinical identification of MDR may be the measurement of radiolabeled drug in tumors in vivo following noninvasive administration and external radionuclide imaging.

PET imaging of MDR drugs labeled with positron-emitting radionuclides could be used to predict drug uptake in tumors, identify MDR tumor cells and evaluate potential inhibitors of p-glycoprotein. Our ultimate objective was to develop the use of PET in the planning of chemotherapeutic regimen. Present studies are a prelude to our planned studies of biodistribution as well as identification of MDR resistant tumors in vivo using ^{11}C-CHC and PET.

MATERIALS AND METHODS

Cell Lines

We used the same cell lines and animal tumor models previously established in our laboratory (*10,11*). The present studies were carried out with human neuroblastoma cell line BE(2)-C sensitive to CHC and corresponding resistant cell line BE(2)-C/CHCb selected with 0.2 μg/ml CHC (*15*).

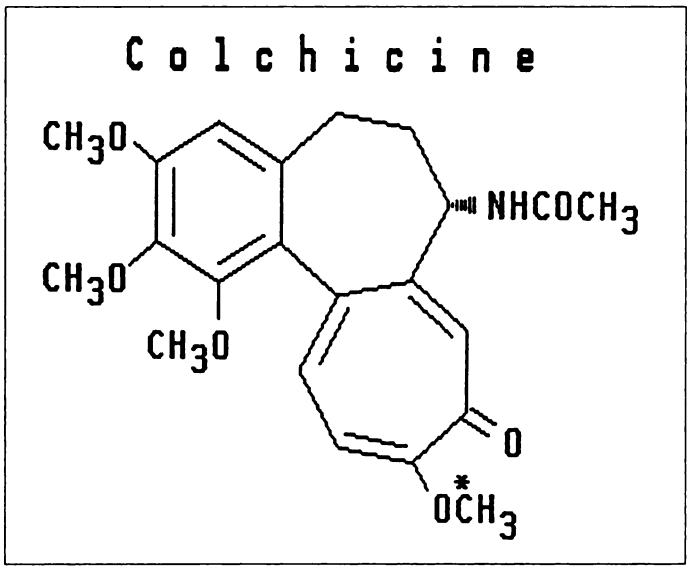

FIGURE 1. Structure of CHC. *Ring C methoxy group, the suggested site of labeling with ^{11}C.

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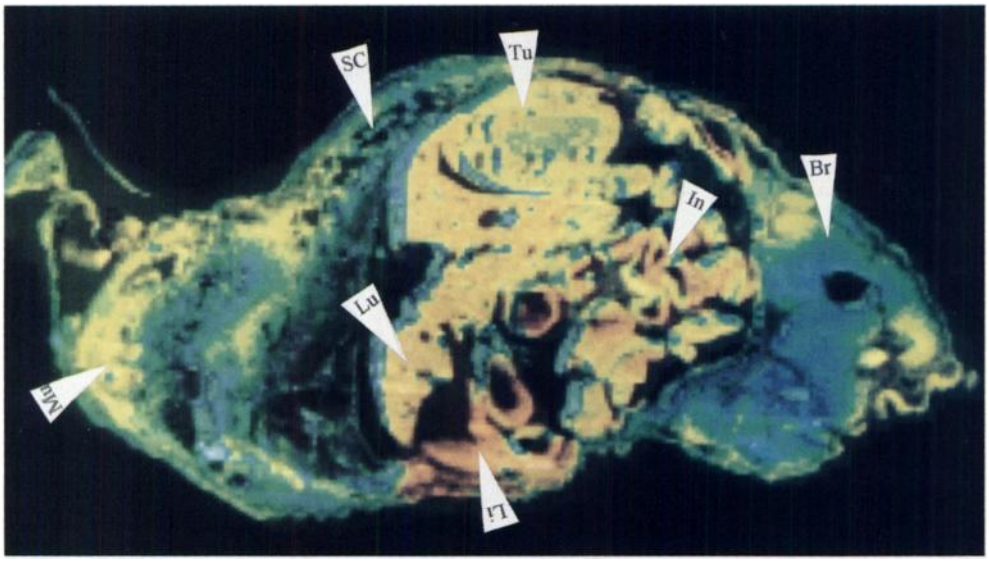

FIGURE 2. Three-dimensional fusion image of comprehensive H & E staining and uptake of ^{14}C-CHC by QAR in sensitive tumor (BE(2)-C) bearing animals. Arrows indicate as marked: Tu = tumor; Br = brain; In = intestines; Li = liver; Lu = lung Mu = muscle; Sc = spinal chord.

Animals and Whole-Body Sectioning

Two sets of Balb/c nude mice (20–25 g body weight) were xenografted with dorsal subcutaneous injection of 10^7 cells each of CHC-sensitive and -resistant cells. When the tumor grew to 0.5–1.0 g (usually after 14–21 days), the animals were anesthetized with pentobarbital (50 μg/g body weight), and [ring C-methoxy-) ^{14}C-CHC (sp. activity 36.0 mCi/mmole (1.332 GBq/mmole) (New England Nuclear, Inc., Boston, MA); 100 nCi/100 μl per animal (25 g body weight) was injected retro-orbitally. Animals were reanesthetized, if necessary, with pentabarbital as before, and at the end of 60 min were flash frozen in Foraine 12 refrigerant (Atochem North America Inc., Philadelphia, PA) and embedded in OCT 4583 embedding compound (Miles Lab. Diagnostic Division, Elkhart, IN). Using 25 μm, sagittal whole-body sections in increments of 250 μm were made at −20°C with cryomicrotome modified for whole-body sections. The sections were mounted on 75 × 50-mm glass slides and immediately transferred to a freezer at −20°C.

Quantitative Autoradiography

After storing the whole-body sections for 7–10 days the slides were mounted on a Bainbridge Board along with ^{14}C standards (Amersham, MA) and exposed to SB-5 Kodak x-ray film (Kodak, Rochester, NY) for approximately 8–9 days at −20°C. No enhancing screens were used. Completed microautographs were developed using standard methodology. Autoradiography sections and standards were digitized and the mean radioactivity in tumor and nontumor tissues calculated in nanomoles per gram of tissue weight by comparing them to a set of standards containing known amounts of ^{14}C radioactivity.

Comparison of Carbon-14-CHC Tumor Uptake and Histochemistry

Autoradiography and H & E sections from sensitive and resistant tumor-bearing animals were digitized by a video camera via an image capture board onto a computer. These images were then transferred to a DEC micro VAX (Maynard, MA) where surface contours of the autoradiography and histochemistry sections were drawn on serially lined sections, enabling matching of the two to take place. These were then displayed separately as well as superimposed with a colorwash display to allow comparison of localization of ^{14}C-CHC to cellular components of tissue stroma.

RESULTS

Figures 2 and 3 show a comprehensive three-dimensional fusion image of H & E staining and the relative uptake of ^{14}C-CHC in BE(2)-C (sensitive) and BE(2)-C/CHCb (resistant) tumor-bearing animals, respectively. Using a three-dimensional fusion imaging technique (*12*), we were able to superimpose both images and obtain a comprehensive picture depicting more accurate tissue penetration of radioactivity. The three-dimensional fusion image shows various major organs along with the tumors of both the sensitive and resistant cell lines.

Table 1 shows the mean relative uptake of ^{14}C-CHC in tumors and nontumor tissues of BE(2)-C (sensitive) and BE(2)-C/CHCb (resistant) tumor bearing animals from the QAR determination as nmole/g.

Fusion images allowed direct comparison of ^{14}C-CHC uptake in tumor and nontumor tissues. Mean ^{14}C-CHC distribution in

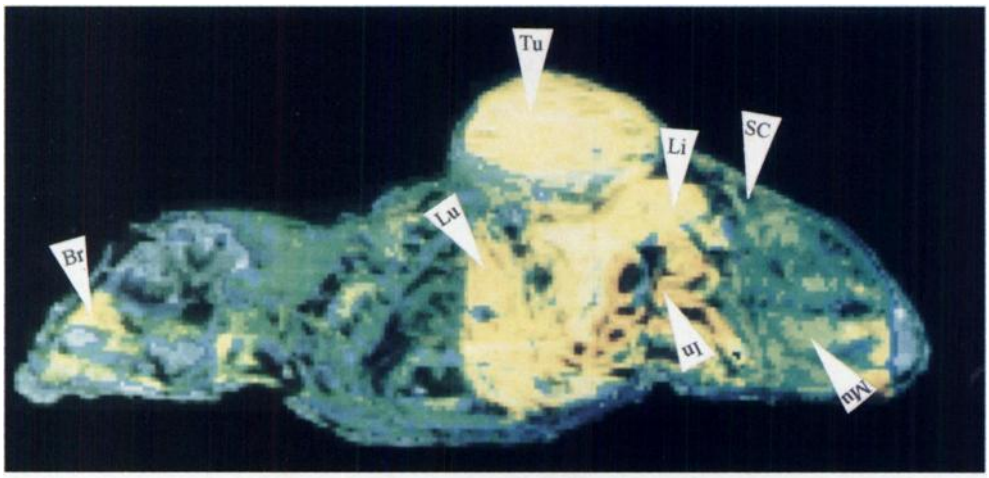

FIGURE 3. Three-dimensional fusion image of comprehensive H & E staining and uptake of ^{14}C-CHC by QAR in resistant tumor (BE(2)-C/CHCb) bearing animals. Arrows indicate as marked: Tu = tumor; Br = brain; In = intestines; Li = liver; Lu = lung Mu = muscle; Sc = spinal chord.

TABLE 1
Tissue Distribution of Carbon-14-CHC 1 Hour Postinjection as Measured by Quantitative Autoradiography

Tissue	BE(2)-C (nmole/g)	BE(2)-C/CHCb (nmole/g)
Tumor	24.48 ± 1.21	11.08 ± 4.38
Liver	63.79 ± 6.61	42.01 ± 7.53
Kidneys	31.44 ± 13.00	54.32 ± 39.30
Intestines	42.98 ± 1.66	50.51 ± 22.83
Brain	6.31 ± 4.44	3.11 ± 1.32
Lungs	12.88 ± 6.31	10.03 ± 0.04
Spinal chord	2.34 ± 0.34	2.14 ± 0.81
Muscle	6.21 ± 1.39	8.63 ± 2.49
Bone	10.89 ± 2.04	8.08 ± 0.89
Heart	36.59 ± 15.26	11.71 ± 0.73

sensitive and resistant tumors was 24.48 ± 1.21 (0.92% ± 0.05 %ID) and 11.08 ± 4.38 (0.42 ± 0.16 %ID) nmoles/g ($p < 0.001$) respectively with nontumor tissue distribution in both groups being similar. The results agree with our earlier studies with ^{14}C-CHC (*11*). Possible metabolism of CHC and the relative tumor-to-plasma ratios were discussed earlier in our studies with ^{14}C-CHC (*11*). Sixty to 70% of the radioactivity content of tumor was present as intact colchicine, and the remainder were low molecular weight metabolites which were in equilibrium with the plasma and extracellular fluid component.

DISCUSSION

Since BE(2)-C/CHCb tumor cells are multidrug resistant, the retention of ^{14}C-CHC in vivo was expected to be less in BE(2)-C/CHCb than in BE(2)-C tumors. This is proven by our data, which show ^{14}C-CHC concentrations more than twice in sensitive than in resistant tumors. The observed systemic distribution of the ^{14}C label is consistent with previous reports on Colchicine behavior in vivo. The high concentrations of activity in liver and intestine are in accord with the accepted detoxification of CHC via the bile (*16,17*). The relative exclusion from brain of radioactivity may be explained by the presence of elevated p-glycoprotein expression in cerebral capillary endothelial cells (*18–20*).

The present study demonstrates the possibility of distinguishing multidrug resistant from sensitive tumors in vivo using a radiolabel MDR drug. Three-dimensional fusion imaging of H & E staining and QAR has further emphasized the fact that whole-body imaging can be carried out with radiopharmaceuticals such as ^{14}C-CHC. This, in principle, suggests the possibility of monitoring MDR in patients by PET. To this end, we have successfully formulated a ^{11}C-analogue of CHC. The evidence provided by the present data also establishes the possibility of using CHC as the drug of choice for PET imaging in patients.

CONCLUSION

Three-dimensional QAR has potential application in biodistribution studies. Moreover, use of ^{11}C-CHC to identify MDR tumors in patients may be possible.

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