In Vitro Uptake of Technetium-99m-Teboroxime in Carcinoma Cell Lines and Normal Cells: Comparison with Technetium-99m-Sestamibi and Thallium-201

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Since ^{201}Tl, ^{99m}Tc-sestamibi and ^{99m}Tc-teboroxime concentrate in cardiac cells through different mechanisms, we compared their uptake in cultured normal cells and carcinoma cell lines in order to define their possible use for tumor evaluation in vivo. Four lines of normal cells from animals, including myocytes from newborn rats, and four lines of human carcinoma cell lines were incubated for 1 hr with 37 kBq of either tracer. Results, expressed in percent of the total activity taken up by 1 million cells, showed a 9% difference between the uptake of teboroxime by normal and carcinoma lines (24.6% ± 2.8% versus 22.5% ± 2.1%, respectively, $p < 0.05$). Mean uptake was 80% higher in tumor than in normal cells for ^{201}Tl (5.39% ± 1.33% versus 3.00% ± 1.08%, respectively, $p < 0.001$) and nearly 4 times higher for sestamibi (5.37% ± 2.34% versus 1.44% ± 1.88%, $p < 0.001$). For both agents, uptake by the myocytes and carcinoma cells was comparable (5.14% ± 0.11% for ^{201}Tl and 5.28% ± 1.03% for sestamibi). When the myocytes are excluded from the group of normal cells, the uptake is 112% higher in tumor than in normal cells for ^{201}Tl (5.39% ± 1.33% versus 2.54% ± 0.44%, $p < 0.001$) but it becomes nearly nine times higher for sestamibi (5.37% ± 2.34% versus 0.60% ± 0.23%, $p < 0.001$). It is concluded that these experiments show that the uptake of sestamibi was the most discriminant to separate between normal and malignant cells, while teboroxime was the less discriminant. Potential clinical applications for tumor visualization based on differences in sestamibi and teboroxime uptake could be envisioned.

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The three blood flow imaging agents currently available for routine clinical applications, i.e., ^{201}Tl-chloride, ^{99m}Tc-sestamibi and ^{99m}Tc-teboroxime, concentrate in cells through different mechanisms. Thallium-201 mostly follows the potassium pathway through the ATPase-dependent Na^+/K^+ pump (*1*), whereas sestamibi is driven into the mitochondria by its transmembrane potential (*2*), and teboroxime possibly binds to the plasmic membrane (*3*). Following a bolus injection, the myocardial uptake of these three agents closely correlates with coronary artery blood flow (*4*). Clinically, an increased uptake of ^{201}Tl and sestamibi has been noted in several kinds of tumors (*5–18*). Recent in vitro experiments in cultured human carcinoma cells have demonstrated that sestamibi uptake is largely increased when compared with nontumorous cell lines (*19*). The purpose of the present work was to assess the cellular uptake of teboroxime in several lines of normal and tumor cells and to compare it with the uptake of ^{201}Tl and sestamibi in similar conditions. We have observed that teboroxime uptake remains fairly constant in all types of cells, while ^{201}Tl and sestamibi uptake vary widely between these cells lines.

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MATERIALS AND METHODS

Cell Cultures

The normal cells were rabbit skin fibroblasts, neonatal rat skin fibroblasts, neonatal rat heart fibroblasts and neonatal rat myocytes. The tumor cells were human melanoma cells with either a high or a low melanin content, human colic carcinoma cells and human breast carcinoma cells.

Myocytes and cardiac fibroblasts were prepared according to a method formerly described (*3*) and derived from Harary (*20*). Briefly, the hearts of 2–4-day-old Sprague-Dawley rats are removed and the ventricles are isolated and trypsinized. The myocytes are separated from the nonmuscular cells by the differential attachment technique (*21*). The myocytes, attaching less rapidly to the surface of the culture dish than the nonmuscular cells, are washed away after 3 hr and replated in a different culture dish. The final plating of these myocytes is done by placing around 650,000 cells bathed in 2 ml of 10% fetal calf serum into 35-mm diameter culture dishes. After 3 days in culture, confluent monolayers of nearly 1 million cells per dish are obtained with at least 80% of synchroneously beating cells. The fibroblasts are cultured into 75-cm^2 culture dishes for 3–5 passages and finally plated into 35-mm diameter culture dishes. All dishes were kept at 37°C in a 95% air, 5% CO_2, water-saturated atmosphere.

The skin fibroblasts from rabbits and neonate rats were obtained from skin explants maintained until overgrowth in a nutrient medium containing 20% fetal calf serum. The explants were

discarded and the cells growing out from the explants were cultured in a medium containing 10% fetal calf serum.

The human melanoma cell lines were derived from metastatic tumors. All were obtained through another laboratory (INSERM U218, Lyon, France). The code names for these cell lines are M3Dau for those with the low melanin content, and M4Beu for those with a high melanin content. The human colon and breast carcinoma lines, respectively the MCF7 and SW948 lines, were obtained through a different laboratory (Sandoz Forschungs Institute, Vienna, Austria). Following defrosting, the cells were proliferated in 75-cm^2 dishes for 3 days and submitted to three 3-day cycles of trypsinization-replating. Final plating was performed in 10-cm^2 dishes with a density of 200,000 cells per dish. After 3 days in culture, only the M4Beu cells formed a complete monolayer, while the others, particularly the SW948 cells, were partly aggregated in colonies. Counting of these cells, performed after trypsinization, showed a density ranging between 1.0 and 1.4 million cells per dish.

Tracer Preparation

A 370-kBq/ml ^{201}Tl solution was obtained by diluting a 37-MBq/ml preparation of thallous chloride (CIS Bio International, Gif-sur-Yvette, France). Technetium-99m-sestamibi (DuPont Pharma SA, Paris, France) and ^{99m}Tc-teboroxime (Bristol-Myers Squibb, New Brunswick, NJ) were prepared according to the manufacturers' instructions and 370-kBq/ml solutions were obtained.

Uptake Measurements

Thirty minutes before adding the tracers, the incubation medium was replaced by 2 ml of serum-free medium. If necessary, the pH was readjusted at 7.4.

A 37-kBq dose of either tracer in 0.1 ml was added to the incubation medium and the cells were incubated in the same environmental conditions for 1 hr, with at least five dishes per cell line. This was enough time for the three tracers to achieve equilibrium. The dishes were then rapidly washed three times (total time <10 sec) with a 4°C saline solution. The bottom wall of the dish was separated from the lateral wall and its activity was counted in a gamma-well counter. The number of cells per dish was counted on a hemocytometer in five culture dishes with the same preparation for each type of cell.

Results are presented as the percentage of the activity added in each dish per million cells and expressed as mean ± s.d. Statistical comparisons were done using the Student's t-test for unpaired data.

RESULTS

Cellular uptake of ^{201}Tl is shown in Figure 1. The mean uptake in the normal cells was 3.00% ± 1.08% versus 5.39% ± 1.33% in the tumor lines ($p < 0.001$). There were large variations among both categories and a significant overlap between the uptake of cell lines of the two groups.

Figure 2 demonstrates that the uptake of sestamibi by nontumorous cells other than myocytes was much lower than the uptake of ^{201}Tl in the same lines (2.54% ± 0.44% for ^{201}Tl versus 0.60% ± 0.23% for sestamibi, $p < 0.001$). Uptake of both tracers was similar in the myocytes (5.14% ± 0.11% for ^{201}Tl versus 5.28% ± 1.03% for sestamibi, ns), as well in the tumor cell lines (5.39% ± 1.33% versus 5.37% ± 2.34%, ns). Uptake of sestamibi in myocytes was higher than the uptake of three of the four carcinoma cell lines.

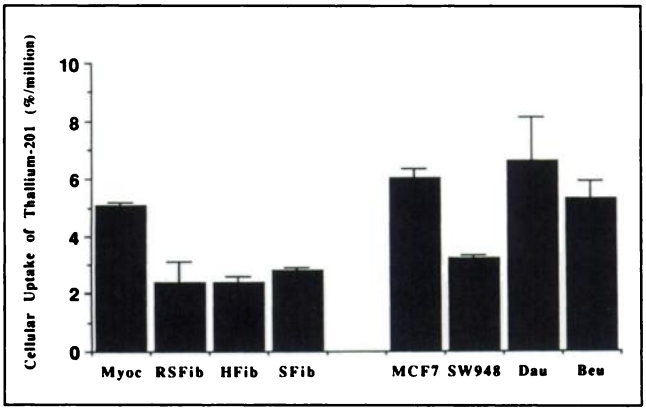

FIGURE 1. Cellular uptake of ^{201}Tl expressed as percent of the total dose added per dish per million cells in four normal cell lines (Myoc = newborn rats myocytes; RSFib = skin fibroblasts from rabbits; HFib = heart fibroblasts from newborn rats; SFib = skin fibroblasts from newborn rats) and in four human tumor cell lines (MCF7 = colon carcinoma; SW948 = breast carcinoma; Dau = melanoma cells with high content of melanin; Beu = melanoma cells with low content of melanin).

Results obtained with teboroxime are shown in Figure 3. Mean uptake was 8% higher in normal cells than in tumor lines (24.6% ± 2.8% versus 22.5% ± 2.1%, respectively, $p < 0.05$), but none of the variations between cell lines as observed with either ^{201}Tl or sestamibi occurred with this tracer.

DISCUSSION

Even in this limited number of cells lines, it appears that teboroxime uptake remains fairly constant with all types of cells, while ^{201}Tl uptake shows a moderate degree of specificity for the carcinoma cells. Sestamibi uptake was very low in the fibroblasts but high in the carcinoma cells and in

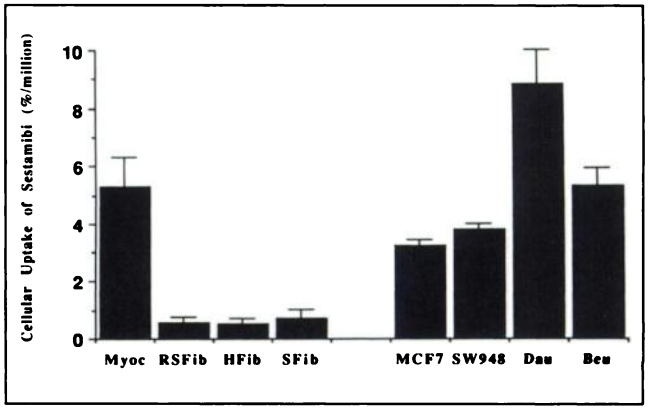

FIGURE 2. Cellular uptake of sestamibi expressed as percent of the total dose added per dish per million cells in four normal cell lines (Myoc = newborn rats myocytes; RSFib = skin fibroblasts from rabbits; HFib = heart fibroblasts from newborn rats; SFib = skin fibroblasts from newborn rats) and in four human tumor cell lines (MCF7 = colon carcinoma; SW948 = breast carcinoma; Dau = melanoma cells with high content of melanin; Beu = melanoma cells with low content of melanin).

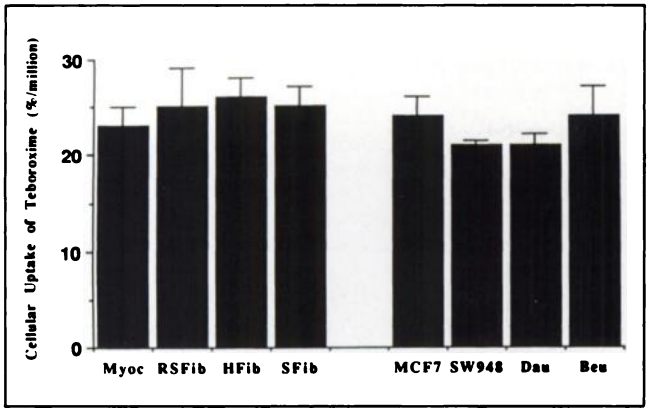

FIGURE 3. Cellular uptake of teboroxime expressed as percent of the total dose added per dish per million cells in four normal cell lines (Myoc = newborn rats myocytes; RSFib = skin fibroblasts from rabbits; HFib = heart fibroblasts from newborn rats; SFib = skin fibroblasts from newborn rats) and in four human tumor cell lines (MCF7 = colon carcinoma; SW948 = breast carcinoma; Dau = melanoma cells with high content of melanin; Beu = melanoma cells with low content of melanin).

the myocytes. Clinical applications of these properties could be envisioned to separate the blood flow component from the hypermetabolism component on the scintigraphic image of a tumor obtained with these agents.

Cellular Uptake of the Tracers

Sestamibi is a cationic agent with modest lipophilic properties that migrate in a nonmediated manner through the sarcolemmal and mitochondrial inner membranes, driven by a cascade of negative transmembrane potentials. Recent data suggest that 90% of the tracer activity is concentrated in the mitochondria (*2*). Since the maintenance of these potentials is directly under the dependence of energy consuming biochemical reactions, it is not surprising that impairment of the main metabolic pathways leading to the production of ATP results in a decreased uptake of this tracer. Similarly, it seems natural that cells with a high content in mitochondria, such as myocytes, concentrate more sestamibi than the more quiescent cells of the fibroblast family (*22,23*). The fact that the carcinoma cell lines concentrate sestamibi like myocytes rather than like fibroblasts is also not surprising. In fact, similar results have been obtained by Delmon-Moingeon et al. (*19*), but they used different cell lines for the tumorous and normal cells. After an incubation time identical to the one we used (1 hr), they found that uptake in the normal cells was <2% of the activity in the medium, while it varied between 5% and 28% for the human tumor cells lines. By modifying the transmembrane potentials through various chemical interventions, they demonstrated that the increased uptake observed in the tumor cells could be accounted for by the presence of these potentials. Increased sestamibi uptake in tumor cells is also supported by several clinical observations reported in the literature regarding tumor localization in the lungs (*11*), brain (*12*), bone and lymph nodes (*13–15*) and the thyroid (*16,17*).

Teboroxime is a highly lipophilic molecule with a high extraction coefficient in the heart (*4*) but a low retention time. In previous experiments with cultured cardiac cells, we observed that cellular uptake of this compound is resistant to metabolic inhibition induced by cyanide and iodoacetate, suggesting a possible passive binding to the membrane and possibly to some proteins (*3*). Even in cells killed by osmotic lysis, uptake was not significantly affected, while under the same conditions uptake of ^{201}Tl and sestamibi were nearing zero. Since the uptake of this tracer is not modified by an impaired metabolism, it is not surprising that it is not modified by an increased metabolism, as expected in carcinoma cells.

The possible role of nonspecific binding of teboroxime to the dish was eliminated by counting only the bottom of the dishes, which are almost entirely covered by the monolayer with most cell lines. Another proof that the measured uptake was only due to the cellular activity is that when the evolution of this activity is measured against time in dishes containing a monolayer of cells, the counted activity keeps increasing for up to 1 hr, while in empty dishes a plateau is attained in a few minutes.

The tumoral concentration of ^{201}Tl has been known nearly from its introduction in nuclear medicine (*5*). Since then, it has been recognized in brain (*6,8,9*), thyroid (*7*), breast, lung cancers and lymphoma (*10*). Its uptake mechanism is mostly flow-dependent, but the ATPase-dependent Na^+/K^+ pump also plays a significant role (*10*).

Limitations

The results of this work are limited by the number of cell lines and species differences. We did not prove that an increased uptake of sestamibi would be observed in all carcinoma cell lines, however, it was the only one of the three agents to show a clear contrast between normal and malignant cells. Conclusions similar to ours were reached by Delmon-Moingeon et al. (*19*) using nine human tumor cell lines and two normal lines. Moreover, several clinical studies have also confirmed the high tumoral uptake of this tracer, although the participation of the blood flow component in these images has never been assessed.

The precise intracellular localization of sestamibi and teboroxime is not clearly demonstrated. Maneuvers modifying the plasma and mitochondrial membrane potentials can modify sestamibi uptake, but the abundance of mitochondria in tested tumor cells has not been determined.

Also lacking in this study are data about the uptake and release kinetics of these tracers. The in vivo kinetics of sestamibi and teboroxime are very different: sestamibi shows a neglectible redistribution during the first hours following administration, while teboroxime is rapidly cleared from the heart. Comparative in vitro data are available only for myocytes (*3*).

Clinical Implications

The most striking finding of the present study is the nearly constant cellular uptake of teboroxime, irrespective of cell type (species or degree of malignancy). Conse-

quently, it can be assumed that its uptake in vivo is only a function of blood flow in normal tissues and in tumors.

We have confirmed that sestamibi uptake varies widely between cell lines, probably in relation with the level of metabolic activity, although this parameter has not been estimated. These measurements have been performed after 1 hr of incubation, which is sufficient time for this tracer to attain equilibrium (*3*). In vivo experimentation is different because most of the tissular activity is extracted during the first pass of the bolus that follows intravenous injection. We have not checked if differences in the rate of uptake also occur in the early phase of incubation in cultures. This is a difficult task with this model because it involves low levels of activity and minute differences in experimental conditions can severely influence the results. Another limitation for in vivo extrapolation is the lack of data regarding the role of tracer uptake by the tumor vascular endothelium. If this was to occur, different permeabilities of tumor endothelium at different stages of tumor growth could possibly impact tumor imaging with sestamibi.

In conclusion, these results suggest that a comparison of teboroxime and sestamibi uptake in tumors could allow possible estimation of the relative participation of blood flow and metabolism in these tumors.

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REFERENCES

1. McCall D, Zimmer LJ, Katz AM. Kinetics of thallium exchange in cultured rat myocardial cells. *Circ Res* 1985;56:370–376.
2. Carvalho PA, Chiu ML, Kronauge JF, et al. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* 1992;33:1516–1521.
3. Maublant JC, Moins N, Gachon P, Renoux M, Zhang Z, Veyre A. Uptake of ^{99m}Tc-teboroxime in cultured myocardial cells: comparison with ^{201}Tl and ^{99m}Tc-sestamibi. *J Nucl Med* 1993;34:255–259.
4. DiRocco RJ, Rumsey WL, Kuczynski BL, et al. Measurement of myocardial blood flow using a co-injection technique for technetium-99m-teboroxime, technetium-99m-sestamibi and thallium-201. *J Nucl Med* 1992;33:1152–1159.
5. Cox PH, Belfer AJ, Van du Pompe WB. Thallium-201 chloride uptake in tumours, a possible complication in heart scintigraphy. *Br J Radiol* 1976; 49:767–768.
6. Ancri D, Basset JY, Lonchampt MF, Etavard C. Diagnosis of cerebral lesions by thallium-201. *Radiology* 1978;128:417–422.
7. Senga O, Miyakawa M, Shirota H. Comparison of ^{201}Tl-chloride and ^{67}Ga-citrate scintigraphy in the diagnosis of thyroid tumor: concise communication. *J Nucl Med* 1982;23:225–228.
8. Kaplan WD, Takvorian T, Morris JH, et al. Thallium-201 brain tumor imaging: a comparative study with pathologic correlation. *J Nucl Med* 1987;28:47–52.
9. Brismar T, Collins VP, Kesselberg M. Thallium-201 uptake relates to membrane potential and potassium permeability in human glioma cells. *Brain Res* 1989;500:30–36.
10. Sehweil AM, McKillop JH, Milroy R, et al. Mechanism of ^{201}Tl uptake in tumours. *Eur J Nucl Med* 1989;15:376–379.
11. Hassan IM, Sahweil A, Constantinides C, et al. Uptake and kinetics of ^{99m}Tc-hexakis 2-methoxy isobutyl isonitrile in benign and malignant lesions in the lungs. *Clin Nucl Med* 1989;14:333–340.
12. O'Tuama LA, Packard AB, Treves SD. SPECT imaging of pediatric brain tumor with hexakis (methoxyisobutylisonitrile) technetium (I). *J Nucl Med* 1990;31:2040–2041.
13. Caner B, Kitapçi M, Aras T, et al. Increased accumulation of hexakis (2-methoxyisobutylisonitrile) technetium (I) in osteosarcoma and its metastatic lymph nodes. *J Nucl Med* 1991;32:1977–1978.
14. Aktolun C, Bayhan H, Celasum B, Kir MK. Unexpected uptake of technetium-99m hexakis-2-methoxy-isobutylisonitrile in giant lymph node hyperplasia of the mediastinum (Castleman's disease). *Eur J Nucl Med* 1991; 18:856–859.
15. Caner B, Kitapçl M, Unlu M, et al. Technetium-99m-MIBI uptake in benign and malignant bone lesions: a comparative study with technetium-99m-MDP. *J Nucl Med* 1992;33:319–324.
16. Balon HR, Fink-Bennett D, Stoffer SS. Technetium-99m-sestamibi uptake by recurrent Hurtle cell carcinoma of the thyroid. *J Nucl Med* 1992;33:1393–1395.
17. Scott AM, Kostakoglu L, O'Brien JP, et al. Comparison of technetium-99m-MIBI and thallium-201-chloride uptake in primary thyroid lymphoma. *J Nucl Med* 1992;33:1396–1398.
18. Khalkhali I, Mena I, Jouanne E, et al. Breast cancer detection with ^{99m}Tc-sestamibi prone imaging: its correlation with mammography and pathology [Abstract]. *Clin Nucl Med* 1992;17:761.
19. Delmon-Moigeon LI, Piwnica-Worms D, Van den Abbeele AD, Holman BL. Uptake of the cation hexakis (2-methoxy-isobutyl-isonitrile)-technetium-99m by human carcinoma cell lines in vitro. *Cancer Res* 1990;50:2198–2202.
20. Harary I, Farley B. In vitro studies of single isolated beating heart cells. *Science* 1960;130:1674–1675.
21. Blondel B, Roijen I, Cheneval JP. Heart cells in cultures: a simple method for increasing the proportion of myoblasts. *J Exp Med* 1970;27:356–358.
22. Chiu ML, Kronauge JF, Piwnica-Worms D. Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium (I) in cultured mouse fibroblasts. *J Nucl Med* 1990; 31:1646–1653.
23. Caldwell JH, Mertens H, Linssen MC, van der Vusse GJ, Buell U, Kammermeier H. Uptake kinetics of technetium-99m-methoxyisobutylisonitrile and thallium-201 in adult rat heart endothelial and fibroblast-like cells in comparison to myocytes. *J Nucl Med* 1992;33:102–107.