Abstract

\(^{99m}\)Tc-methoxyisobutyl isonitrile (MIBI) is a suitable transport substrate for the multidrug resistance gene product \(P\)-glycoprotein (\(P\)-gp) and widely used for tumor imaging. Bromocriptine has been shown to inhibit the ATPase activity and the function of \(P\)-gp. We hypothesized that bromocriptine could promote the accumulation of MIBI by inhibiting \(P\)-gp activities, a feature that can be taken advantage of for enhancing \(^{99m}\)Tc-MIBI imaging. In the current study, we sought to investigate whether bromocriptine enhanced the uptake of \(^{99m}\)Tc-MIBI in hepatocellular carcinoma patients. Sixty primary hepatocellular carcinoma patients received \(^{99m}\)Tc-MIBI single photon emission computer tomography (SPECT) prior to surgery. \(^{99m}\)Tc-MIBI SPECT was performed 15 and 120 min after injection of 20 mCi \(^{99m}\)Tc-MIBI, and early uptake, delayed uptake (L/Nd), and washout rate (L/Nwr) of \(^{99m}\)Tc-MIBI were obtained. In addition, a second \(^{99m}\)Tc-MIBI SPECT was performed according to the same method 48 h after bromocriptine administration. We found that, prior to bromocriptine administration, significant MIBI uptake in tumor lesions was noted in only 10 (16.7%, 10/60) patients with hepatocellular carcinoma. No significant MIBI uptake was observed in the tumor lesions of the remaining 50 (83.3%, 50/60) hepatocellular carcinoma patients. Following bromocriptine administration, all the patients without apparent MIBI uptake demonstrated significant MIBI uptake on \(^{99m}\)Tc-MIBI SPECT (\(P < 0.05\)). Our findings indicate that bromocriptine enhances the uptake of \(^{99m}\)Tc-MIBI in patients with hepatocellular carcinoma.

Keywords: liver cancer, multidrug resistance, \(P\)-glycoprotein, \(^{99m}\)Tc-MIBI, bromocriptine

INTRODUCTION

Until now, the main obstacle to a successful cure of cancer has been the intrinsic or acquired resistance of the neoplastic cells to a variety of structurally and functionally heterogeneous anticancer agents, which is named multidrug resistance (MDR). \(P\)-glycoprotein (\(P\)-gp) is a multidrug resistance gene product that is found on the surface of multidrug resistant cancer cells and is implicated in multidrug resistance\(^{[1-2]}\). As an energy dependent drug efflux pump, the protein reduces the accumulation of chemotherapeutic drugs in multidrug resistant cells.\(^{[10]}\) Therefore, a key step in overcoming multidrug resistance is by inhibition of multidrug resistance-1 (MDR1)/\(P\)-gp expression in hepatocellular carcinoma cells. Various approaches

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have been attempted to block MDR1 overexpression.[4–6] Multidrug resistance modulators capable of blocking P-gp-mediated drug efflux have been suggested to reverse P-gp-mediated drug resistance and to improve the outcome of cancer chemotherapy. They include the anti-arrhythmic drug verapamil and the immunosuppressant cyclosporine, and the second-generation multidrug resistance modulators PSC833 and MS209. However, clinical application of these drugs is hampered by their side effects and the possibility of inhibiting other transporters that are not related to multidrug resistance.

99mTc-methoxyisobutyl isonitrile (MIBI) is a cationic lipophilic agent and widely used for myocardial perfusion imaging and detection of various tumors.[10–16] Recent studies have revealed that 99mTc-MIBI is a suitable transport substrate for P-gp and thus may provide additional information about the P-gp status of tumor cells.[17,18]. MIBI is accumulated within the mitochondria and cytoplasm and malignant tumors show increased transmembrane potential as a result of increased metabolic requirements that induce increased accumulation of MIBI.[19] 99mTc-MIBI imaging has the advantage of noninvasively detecting the presence of P-gp overexpression in vivo.[20–21]. In our previous study, we have found that 99mTc-MIBI is useful for noninvasively detecting the expression of P-gp in hepatocellular carcinoma.[22].

Bromocriptine is a hydrophobic polycyclic molecule and a D2 dopaminergic receptor agonist and has previously been reported to inhibit the ATPase activity and the function of P-gp.[23]. The drug has been used to treat hyperprolactinemia, acromegaly, and Parkinson’s disease for more than two decades and is associated with slight side effects.[24,25]. However, there has been no report on the use of bromocriptine in reversing multidrug resistance in cancer in humans. We speculate that bromocriptine could promote the accumulation of MIBI, a transport substrate for P-gp, by inhibiting P-gp activities, a feature that can be taken advantage of for enhancing 99mTc-MIBI imaging. In the current study, we compared the visualization rate by 99mTc-MIBI imaging of hepatocellular carcinoma patients before and following administration of bromocriptine.

**SUBJECTS AND METHODS**

**Subjects**

We reviewed the clinicopathological and radiological data of consecutive chemotherapy-naïve patients with pathologically proven hepatocellular carcinoma who sought surgical treatment between January 2006 and December 2009 at the Hepatic Center of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. All patients underwent physical examination, abdominal ultrasonography and computerized tomography (CT). The protocol was approved by the local institutional review board and patient consent was not required because of the retrospective nature of the study. All patients underwent 99mTc-MIBI SPECT prior to surgery.

**99mTc-MIBI SPECT**

Liver imaging was performed with a double-head gamma camera equipped with a high-resolution parallel-hole collimator (PRISM 2000; Marconi Medical Systems, Cleveland, OH, USA). Images were obtained 15 and 120 min after injection of 20 mCi 99mTc-MIBI. Early and delayed SPECT of the liver was performed in all patients. After the first liver SPECT, patients were required to take 2.5 mg bromocriptine (Novartis, Basel, Switzerland) orally 3 times per d for 3 d. Thereafter, the second liver SPECT was performed. For SPECT of the liver, 72 projections were obtained using a 64 × 64 matrix at 45 s per view. Image reconstruction was performed using filtered back projection with Butter-worth and ramp filters. Transverse, coronal, and sagittal sections were reconstructed. Attenuation correction was not applied. SPECT images were compared with liver CT images, and accumulation in liver tumors was interpreted independently by two nuclear medicine physicians who were blind to the clinicopathological data of patients. Disagreements were resolved by consensus, with a third observer as referee. The findings on 99mTc-MIBI liver imaging were evaluated semi-quantitatively. Regions of interest (ROIs) were manually defined on the transaxial tomograms with the lesion’s highest uptake in the middle of the tumor. The ROIs placed on the lesions (L) encompassed all pixels that had uptake values of >90% of the maximum uptake in that slice, and the average counting rate in each ROI was calculated. Another ROI of the same size was then drawn over the normal liver (N) on the same transverse section. The early uptake (L/Ne) and the delayed uptake (L/Nd) were obtained. The washout rate (L/Nwr) was calculated using the following formula: L/Nwr = (L/Ne-L/Nd)×100 (L/Ne).

**Statistical analysis**

The data for L/Ne, L/Nd, and L/Nwr was expressed as mean ± SD. Student’s t test was used to evaluate the significance of differences. A P value of 0.05 or less was considered to be significant.
RESULTS

Patient demographic and disease characteristics

Sixty patients with hepatocellular carcinoma were included in the study, including 24 female and 36 male patients with a mean age of 60 ± 11.5 years (range, 30-73 years). Fifty-two (86.7%, 52/60) patients were hepatitis B surface antigen positive, 3 (5.0%, 3/60) were anti-hepatitis C virus antibody positive, and the remaining patients had no known cause of hepatocellular carcinoma. The tumor size of these patients ranged from 1.5 to 15.0 cm.

Bromocriptine enhances the uptake of 99mTc-MIBI in patients with hepatocellular carcinoma

Analysis of 99mTc-MIBI SPECT imaging data of these hepatocellular carcinoma patients revealed that, prior to bromocriptine administration, significant MIBI uptake in tumor lesions was noted in only 10 (16.7%, 10/60) patients with hepatocellular carcinoma. No significant MIBI uptake was observed in the tumor lesions of the remaining 50 (83.3% 50/60) hepatocellular carcinoma patients. Following bromocriptine administration, all the patients without apparent MIBI uptake demonstrated significant MIBI uptake on 99mTc-MIBI SPECT (P < 0.05) (Fig. 1).

DISCUSSION

P-gp, as a drug efflux pump, extrudes 99mTc-MIBI and other drugs from the cells[23,24]. In animal models and clinical studies, faster clearance of 99mTc-MIBI was observed in tumors that expressed P-gp than those that did not[25-27]. It was also found that 99mTc-MIBI L/Nwr values from treatment naïve breast cancers overexpressing P-gp were 2.7 times higher than those not expressing P-gp[20]. In our earlier study, we have found that P-gp expression was significantly higher in those patients with no apparent 99mTc-MIBI uptake compared with those with significant uptake[22].

There have been numerous attempts to restore chemosensitivity to various chemotherapeutic drugs in recalcitrant cancer cells with MDR[20-31]. These strategies have so far remained unsuccessful because of considerable side effect. Bromocriptine, a classical D2 dopaminergic receptor agonist, has been reported to inhibit the ATPase activity and the function of P-gp[7]. The drug has been used to treat hyperprolactinemia and Parkinson’s disease and has exhibited mild side effects[32-35]. Our previous results showed that bromocriptine blocked P-gp-mediated drug resistance in HepG2-MDR cells[36]. Compared with cyclosporine and verapamil that have been attempted for reversing chemoresistance, bromocriptine has little effect on the cytotoxicity of anticancer drugs.

In our current study, we found that bromocriptine could significantly enhance the uptake of 99mTc-MIBI in hepatocellular carcinoma patients who failed to show any noticeable uptake of 99mTc-MIBI. Our findings suggest that bromocriptine as an inhibitor of P-gp activities could be of value as an agent to boost the uptake of 99mTc-MIBI for tumor imaging. It is also tempting to speculate that bromocriptine can be included as part of the therapeutic regimen to overcome chemoresistance of cancer cells. Currently, we are conducting studies on the combination of bromocriptine with other chemotherapeutic agents in postoperative liver cancer patients.

References

[1] Kanzaki A, Takebayashi Y, Ren XQ, Miyashita H, Mori S, Akiyama S, et al. Overcoming multidrug drug resistance in P-glycoprotein/MDR1-overexpressing cell lines by ecteinascidin 743. Mol Cancer Ther 2002; 1: 1327-4.

Fig. 1 MRI and SPECT detection of hepatocellular carcinoma in the right liver. A: MRI image showed that the tumor was located in the posterior lobe of the right liver. B: 99mTc-MIBI SPECT revealed that MIBI accumulated densely at tumor lesion. C: Significant MIBI uptake on 99mTc-MIBI SPECT was noted in tumor lesion after intake of bromocriptine. Arrow indicates the site of tumor lesion.
T, Okumura K. MDR1-mediated interaction of digoxin with antiarrhythmic or antianginal drugs. Biol Pharm Bull 2002; 25: 1604-7.

[3] Labiale S, Gayet L, Martinet E, Rigel D, Baggetto LG. Transcriptional regulation of the human MDR1 gene at the level of the inverted MED-I promoter region. Ann N Y Acad Sci 2002; 973: 468-71.

[4] Kobayashi H, Takemura Y, Wang FS. Retrovirus-mediated transfer of anti-MDR1 hammerhead ribozymes into multidrug-resistant human leukemia cells: screening for effective target sites. Int J Cancer 1999; 81: 944-50.

[5] Meesungnoen J, Jay K, Jay K. Relation between MDR1 mRNA levels, resistance factor, and the efficiency of P-glycoprotein-mediated efflux of pirarubicin in multidrug-resistant K562 sublines. Can J Physiol Pharmacol 2002; 80: 1054-63.

[6] Harris NM, Duffy PM, Crook TJ, Anderson WR, Sharpe P, Hayes MC, et al. Intravascular pH: a potentially important variable affecting efficacy and the further development of anthracycline chemotherapy for superficial bladder cancer. BJU Int 2002; 90: 957-64.

[7] Shiraki N, Okamura K, Tokunaga J, Ohmura T, Yasuda K, Kawaguchi T, et al. Bromocriptine reverses P-glycoprotein-mediated multidrug resistance in tumor cells. Jpn J Cancer Res 2002; 93: 209-15.

[8] Orlowski S, Vakente D, Garrigos M, Ezan E. Bromocriptine modulates P-glycoprotein function. Biochem Biophys Res Commun 1998; 244: 481-8.

[9] Samimi M, Fakhrian R, Mohagheghi M, Dehpour AR. Comparison of the effect of levodopa and bromocriptine on naloxone-precipitated morphine withdrawal symptoms in mice. Hum Psychopharmacol 2000; 15: 95-101.

[10] Vergote J, Moretti JL, Kouroumdjian JC, Garnier-Suilleral A. MRPI1 modulation by PAK-104P: detection with technetium-99m-MIBI in cultured lung tumor cells. Anticancer Res 2002; 22(1A): 251-6.

[11] Zhou J, Higashi K, Ueda Y, Koda M, Guo D, Jisaki F, et al. Expression of multidrug resistance protein and messenger RNA correlate with 99mTc-MIBI imaging in patients with lung cancer. J Nucl Med 2001; 42: 1476-83.

[12] Kim YS, Cho SW, Lee KJ, Hahn KB, Wang HJ, Yim H, et al. 99mTc-MIBI SPECT is useful for noninvasively predicting the presence of MDR1 gene-encoded P-glycoprotein in patients with hepatocellular carcinoma. Clin Nucl Med 1999; 24: 874-9.

[13] Andrews DW, Das R, Kim S, Zhang J, Curtis M. Technetium-MIBI as a glialoma imaging agent for the assessment of multi-drug resistance. Neurosurgery 1997; 40: 1323-32.

[14] Fukushima M, Yoshida D, Hayase N, Kurohara A, Akagi N, Yoshida S. Scintigraphic prediction of resistance to radiation and chemotherapy in patients with lung carcinoma: technetium 99m-tetrofosmin and thallium-201 dual single photon emission computed tomography study. Cancer 1999; 86: 1470-9.

[15] Bom HS, Kim YC, Song HC, Min JJ, Kim JY, Park KO. Technetium-99m-MIBI uptake in small cell lung cancer. J Nucl Med 1998; 39: 91-4.

[16] Cayre A, Cachin F, Maublant J, Mestas D, Feillel V, Ferriere JP, et al. Single static view 99mTc-sestamibi scintimammography predicts response to neoadjuvant chemotherapy and is related to MDR expression. Int J Oncol 2002; 20: 1049-55.

[17] Ramachandran C, Khatib Z, Escalon E, Fonseca HB, Jhabvala P, Medina LS, et al. Molecular studies in pediatric medulloblastomas. Brain Tumor Pathol 2002; 19: 15-22.

[18] Capella LS, Gefe MR, Silva EF, Affonzo-Mitidieri O, Lopes PG, Rumpianek VM, et al. Mechanisms of vanadate-induced cellular toxicity: role of cellular glutathione and NADPH. Arch Biochem Biophys 2002; 406: 65-72.

[19] Vergote J, Moretti JL, de Vries EG, Garnier-Suillard A. Comparison of the kinetics of active efflux of 99mTc-MIBI in cells with P-glycoprotein-mediated and multidrug-resistance phenotypes. Eur J Biochem 1998; 252: 140-6.

[20] Heiba SI, Santiago J, Miraiziehame M, Jana S, Dede F, Abdel-DayDem HM. Transient postischemic stunning evaluation by stress gated TI-201 SPECT myocardial imaging: Effect on systolic left ventricular function. J Nucl Cardiol 2002; 9: 482-90.

[21] Yuksel M, Cermik F, Doganay L, Karlkaya C, Cakir E, Salan A, et al. 99mTc-MIBI SPET in non-small cell lung cancer in relationship with Pgp and prognosis. Eur J Nucl Med Mol Imaging 2002; 29: 876-81.

[22] Wang H, Chen XP, Qiu FZ. Expression of Multidrug Resistance Protein and Messenger RNA Correlate with 99mTc-MIBI Imaging in Patients with Hepatocellular Carcinoma. World J Gastroenterol 2004; 10: 1281-5.

[23] Ak I, Aslan V, Vardareli E, Gulbas Z. Assessment of the P-glycoprotein expression by 99mTc-MIBI bone marrow imaging in patients with untreated leukaemia. Nucl Med Commun 2003; 24: 397-402.

[24] Sasaki M, Kuwabara Y, Ichiya Y, Yoshida T, Nakagawa M, Soeda H, et al. Prediction of the chemosensitivity of lung cancer by 99mTc-hexakis-2-methoxyisobutyl isotininrile SPECT. J Nucl Med 1999; 40: 1770-83.

[25] Del Vecchio S, Zanetti A, Ciarniello A, Aloj L, Caraco C, Fonti R, et al. Dynamic coupling of 99mTc-MIBI efflux and apoptotic pathway activation in untreated breast cancer patients. Eur J Nucl Med Mol Imaging 2002; 29: 809-14.

[26] Tatsumi M, Tsuruo T, Nishimura T. Evaluation of MS-209, a novel multidrug-resistance-reversing agent, in tumour-bearing mice by technetium-99m-MIBI imaging. Eur J Nucl Med Mol Imaging 2002; 29: 280-94.

[27] Kostakoglu L, Guc D, Canpinar H, Kars A, Alper E, Kirtali P, et al. P-glycoprotein expression by technetium-99m-MIBI scintigraphy in hematologic malignancy. J Nucl Med 1998; 39: 1191-7.

[28] Takamura Y, Miyoshi Y, Taguchi T, Noguchi S. Prediction of chemotherapeutic response by Technetium 99m-MIBI scintigraphy in breast carcinoma patients. Cancer
Bromocriptine enhances uptake of \textsuperscript{99m}Tc-MIBI. Case report and review of the literature. \textit{J Neurosurg} 2003; 99: 397-401.

[30] Wu JY, Fong WF, Zhang JX, Leung CH, Kwong HL, Yang MS, \textit{et al.} Reversal of multidrug resistance in cancer cells by pyranocoumarins isolated from Radix Peucedani. \textit{Eur J Pharmacol} 2003; 473: 9-17.

[31] Marian T, Szabo G, Goda K, Nagy H, Szincsak N, Juhasz I, \textit{et al.} \textit{In vivo} and \textit{in vitro} multitracer analyses of P-glycoprotein expression-related multidrug resistance. \textit{Eur J Nucl Med Mol Imaging} 2003; 30: 1147-54.

[32] Psarros T, Zouros A, Coimbra C. Bromocriptine-responsive akinetic mutism following endoscopy for ventricular neurocysticercosis. Case report and review of the literature. \textit{J Neurosurg} 2003; 99: 397-401.

[33] Stecker MM, Myers SM. Reserpine responsive myoclonus and hyperpyrexia in a patient with Angelman syndrome. \textit{Clin Neurol Neurosurg} 2003; 105: 163-7.

[34] Hattori N. Macroprolactinemia: a new cause of hyperprolactinemia. \textit{J Pharmacol Sci} 2003; 92: 171-7.

[35] Yavuz D, Deyneli O, Akpinar I, Yildiz E, Gozu H, Sezgin O, \textit{et al.} Endothelial function, insulin sensitivity and inflammatory markers in hyperprolactinemic premenopausal women. \textit{Eur J Endocrinol} 2003; 149: 187-93.

[36] Hai Wang, Xiaoping Chen, Fazu Qiu. Overcoming Multi-Drug Resistance of HepG2 cells by Bromocriptine. \textit{World J Gastroenterol} 2004; 10: 1281-5.