

Changes of Tc-99m Sestamibi uptake in P-glycoprotein expressing Leukaemia Cells Treated in vivo with Antisense Oligodeoxynucleotide Complementary to *mdr1* mRNA

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Abstract

We examined the feasibility of Tc-99m sestamibi to monitor changes of mRNA expression of MDRI/P-glycoprotein (P-gp) following antisense oligodeoxynucleotide (AS-ODN) treatment in vivo. Three days after the intraperitoneal inoculation of murine leukaemia P388/R cells expressing MDRI/P-gp in CDF1 mice, 15-mer phosphorothioate AS-ODN to the initiation codon of mouse *mdr1* mRNA was administered intraperitoneally at 10 mg/kg daily for 3 or 4 days. Cells collected from ascites were suspended in medium for Tc-99m sestamibi uptake studies. To know the duration of antisense effects, cells were harvested 2 days later after the 3-day treatment. AS-ODN treatment increased Tc-99m sestamibi uptake. Effects of 3-day treatment and 4-day treatment were the same. Treatment effects were not detected when uptake was observed 2 days after 3-day treatment. Based on the results it was concluded that in vivo treatment with AS-ODN specific to the coding portion of *mdr1* mRNA increased Tc-99m sestamibi uptake in leukaemia cells possessing MDR function.

Key Words: Antisense, Oligodeoxynucleotide, mRNA, P-glycoprotein, Tc-99m sestamibi

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Introduction

Multi-drug resistance (MDR) is a major reason for chemotherapeutic failure in malignant tumours. Rapid efflux of cytotoxic drugs by ATP-binding cassette (ABC) transporters such as MDRI/P-glycoprotein (P-gp) and multi-drug resistance associated proteins (MRP) is a major factor contributing to MDR (1). Chemical modulator of P-gp such as verapamil, cyclosporin A, diltiazem, FK-506, PSC-833 and MS-209 have been evaluated to reverse function of transporters. However, clinical use of reversing agents has been limited by their significant toxicity and poor specificity (1). Alternative approach is the translational inhibition of mRNA coding ABC transporters with antisense oligodeoxynucleotides (AS-ODN) or hammerhead ribozymes (1).

Technetium-99m-methoxyisobutylisonitrile (Tc-99m sestamibi) is a substrate of P-gp and MRP; therefore, its cellular accumulation is suppressed in malignant cells that express these transporter proteins. The accumulation is inversely proportional to the level of their expression, based on which Tc-99m sestamibi may be used to monitor changes of their expression level after MDR-reversing therapy. Furthermore, there is sufficient evidence that the content of mRNA for MDR proteins is inversely correlated with Tc-99m sestamibi accumulation in cells.

We have recently found that AS-ODN complementary to *mdr1* mRNA could be targeted in the murine leukaemia cell line, P388/R, that expresses P-gp (2). In the current investigation, Tc-99m sestamibi uptake was assessed in P388/R cells and its parental non-MDR P388 cells in order to determine whether Tc-99m sestamibi could detect changes of P-gp function induced with in vivo AS-ODN treatment to mRNA coding *mdr1*.

Materials and methods

Cell lines

The P388 murine leukaemia cell line and its sub-clonal MDR cell line P388/R were used (Cancer Chemotherapy Centre, Japanese Foundation for Cancer Research, Tokyo, Japan). The P388/R cell line was developed by repeated treatment with doxorubicin in mouse ascites (2). Cells were passaged through female BALB/c X DBA/2 (CDF1) mice (Nihon SLC, Hamamatsu, Japan) by peritoneal injection and harvested 6-7 days after implantation.

AS-ODN Administration

A 15 bp AS-ODN (5'-TCTTCAAACCTCCATC-3'), complementary to the sequences locating at -1 to 14 of *mdr1* mRNA, was obtained commercially (Grana, Tokyo, Japan). This ODN includes the base pairs of the initiation codon and loop forming site, and has been shown to have the specific inhibition of P-gp expression (3). Statistical calculations based on the number of the human genome suggest that minimum ODN size needed to recognize a specific gene is between 12 and 15 bases in size (4). AS-ODN was phosphorothioated from 5' to 3' ends to enhance the resistance against exonuclease and endonuclease digestion. Specific accumulation of this phosphorothioated AS-ODN in P388/R cells was previously confirmed (2).

Mice (6-8 weeks old, 20 g) were intraperitoneally implanted with P388/R cells at 2×10^6 cells/0.2 mL (day 1). Intraperitoneal administration of AS-ODN was initiated 3 days after cell implantation (day 4) at 10 mg/kg/day and continued daily for 3 or 4 days. Dose of AS-ODN was determined according to previous reports (4,5). The same volume of PBS was given to non-treated control mice. On day 6 or 7, the mice were anesthetized with diethyl ether and ascites was harvested. Cells were immediately washed with cold PBS twice, and suspended in RPMI 1640 medium at 6×10^6 cells/mL (Nissui Seiyaku, Tokyo, Japan)

supplemented with 10% fetal calf serum for Tc-99m sestamibi uptake studies. In order to know the duration of antisense effects, cells were harvested 2 days later (day 8) after the 3-day treatment.

Because specificity of AS-ODN to P388/R cells was previously observed (2), observation of treatment effects on P388 cells were omitted in the current investigation. Based on the same reason, treatment with sense ODN was not performed, either.

Tc-99m sestamibi uptake

Tc-99m sestamibi (Daiichi Radioisotope Laboratory, Tokyo, Japan) was prepared with 2 mL of Tc-99m pertechnetate (370 MBq/mL). Aliquots of 150 μ L/tube of cell suspension were prepared and 10 μ L (37 kBq) of diluted Tc-99m sestamibi solution was added. Cell suspensions were incubated at 37°C in a water-saturated atmosphere containing 5% CO₂. Incubation was terminated at various time points by centrifugation at 400 g for 5 min (n=4). Cell pellets were washed with cold PBS twice, and radioactivity of cell pellets were measured with a well-type gamma counter. Tubes containing the culture medium without cells were similarly treated to assess non-specific radioactivity in samples.

Results were expressed as ratios of Tc-99m MIBI uptake in cells treated with AS-ODN to that in control cells treated with PBS. Statistical analysis was performed with one-way analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD). In the analysis, the level of significance was set at 5%.

Results

Tc-99m sestamibi uptake in P388/R cells in the control condition was significantly lower than that in P388 cells at any time point up to 4 hrs, indicating the MDR capability of P388/R cells (Figure 1). This difference was reproducible in

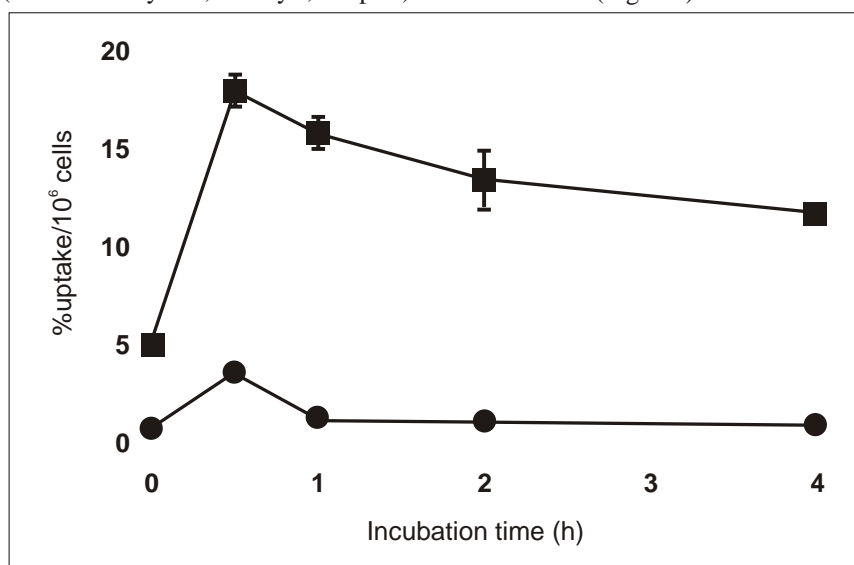


Figure 1. Uptake of Tc-99m sestamibi in P388 murine leukaemia cells (square) and its Sub-clonal MDR P388/R cells (circle) collected from murine ascites (n=4). Bars indicate the s.d., which are omitted when the range is within the symbol.

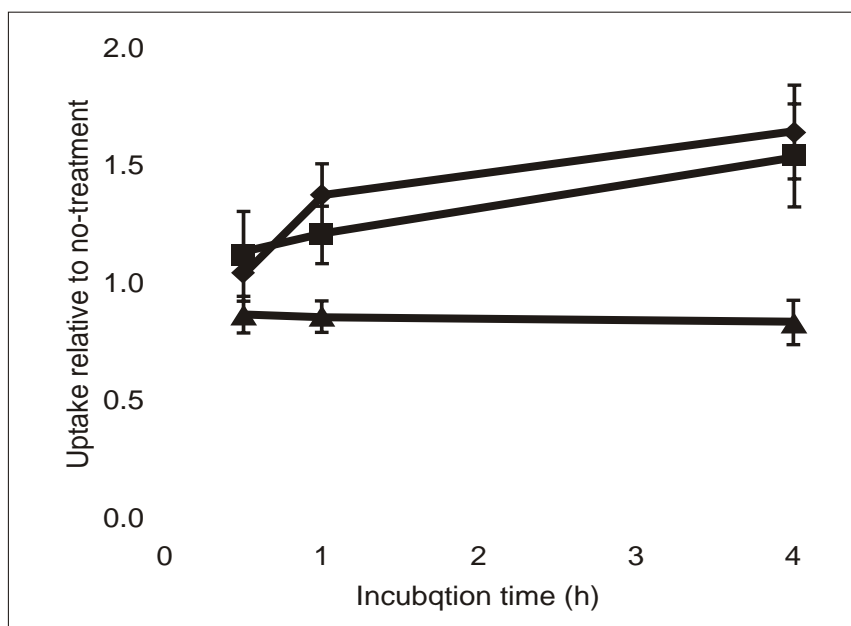


Figure 2. Tc-99m sestamibi uptake in P388/R cells. Cells were harvested from ascites in mice treated with intraperitoneal administration of antisense oligodeoxynucleotide complementary to *mdr1* mRNA at daily doses of 10 mg/kg. Antisense treatment was conducted for 4 consecutive days and uptake study was performed on the last day of treatment (diamond); antisense treatment was conducted for 3 consecutive days and uptake study was performed on the last day of treatment (square); antisense treatment was conducted for 3 consecutive days and uptake study was performed 2 days later after treatment (triangle) (n=4). Bars indicate the s.d. Please note that There was no appreciable difference in the increase between 3-day treatment and 4-day treatment. No significant increase was observed 2 days after 3-day treatment, suggesting recovery of the P-gp expression on cell surface.

all experimental sets, which served as confirmation of P-gp expression of these cell lines throughout this study. AS-ODN treatment markedly increased Tc-99m sestamibi uptake in P388/R cells (Figure 2). There was no appreciable difference in the increase between 3-day treatment and 4-day treatment. No significant increase was observed 2 days after 3-day treatment, suggesting recovery of the P-gp expression on cell surface.

Discussion

The abilities of antisense ODN to suppress P-gp expression level have been evaluated in different experimental models. In vitro study has reportedly shown the complete reversal of mRNA level in antisense ODN-treated P388/R cells. In the current investigation, administration of AS-ODN for up to 4 consecutive days enhanced Tc-99m sestamibi uptake in P388/R cells, but the uptake in P388/R cells was not completely reversed by the treatment in comparison to that in P388 cells. Possible factors that might have limited the effects of ODN treatment would include the following: (a) increase of cell number due to proliferation during treatment, (b) difficulty to keep constant ODN concentration in the peritoneal cavity because of continuous production of ascites by cells and absorption of ODN in the circulation, (c) insufficient cellular

incorporation of naked ODN, and (d) relatively long life-time of P-gp on cell surface.

Life-time of P-gp reportedly varies among cell types from 17 to 72 hrs, suggesting that treatment duration required inhibiting P-gp expression level enough to reflect on reversal effects would vary depending on cell types. P-gp molecules presenting on cell surface may remain after AS-ODN treatment, irrespective of suppression of newly synthesized P-gp, so that the amount of survived P-gp molecules may still be enough to keep Tc-99m sestamibi out of cells. Under such circumstances, longer treatment might be necessary to exert complete reversal. Furthermore, efficient transmembrane delivery of AS-ODN would be required to effectively inhibit P-gp expression. For instance, chemical modifications to neutralize the negative charge of AS-ODN by association with carriers such as cationic lipids improve the cellular uptake of AS-ODN (2).

In summary, this investigation demonstrated the increase of Tc-99m sestamibi uptake in MDR-cells modulated in vivo with AS-ODN treatment that targeted *mdr1* mRNA. This result suggests that in vivo inhibition of mRNA function can be monitored by Tc-99m sestamibi.

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