Antisense imaging of colon cancer-bearing nude mice with liposome-entrapped 99m-technetium-labeled antisense oligonucleotides of c-myc mRNA

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Abstract
AIM: To investigate the feasibility for antisense imaging of the colon cancer with liposome-entrapped 99m m-technetium labeled antisense oligonucleotides as tracers.

METHODS: Fifteen mer single-stranded aminolinked phosphorothioate oligonucleotides of c-myc mRNA were labeled with 99mTc-pertechnetate, then purified and finally entrapped with liposomes to form the labeling compounds, liposome-entrapped 99mTc-labeled antisense oligonucleotides. The LS-174-T cells (colon of adenocarcinoma cell line) were incubated with the labeling compounds to test the uptake rates of LS-174-T cells. Later on, a model of 30 tumor bearing nude mice was constructed by administering liposome-entrapped 99mTc-labeled antisense oligonucleotides. Then some of the tumor bearing nude mice were sacrificed at 0.5, 1, 2, and 4 h after intravenous injection, and proper quantity of liver, spleen, tumor, etc. was obtained. The tissues were counted in a gamma counter, and after correction for decay and background activity, expressed as a percentage of the injected dose. The ratios of radioactive counts in tumor to that in contralateral equivalent region of abdomen were calculated.

RESULTS: The uptake rates of LS-174-T cells for liposome-entrapped 99mTc-labeled antisense oligonucleotides increased as time prolonged and reach the peak (17.77±2.41%) at 7 h. The biodistributions showed that the radioactivity in the tumor (13.46±0.2%) of injected dose was the highest at 2 h of intravenous injection of liposome-entrapped 99mTc-labeled antisense oligonucleotides, and then decreased sharply to 4.58±0.45% at 4 h. The tumor was shown clearly in the whole-body scan at 2 h of intravenous injection. The ratios, radioactive counts in tumor to that in contralateral equivalent region of abdomen (1.7332±0.2537), was the highest one at 2 h after intravenous injection of liposome-entrapped 99mTc-labeled antisense oligonucleotides.

CONCLUSION: The liposome-entrapped 99mTc-labeled antisense oligonucleotides deserve being developed into radiopharmaceutics for the colon cancer imaging.

INTRODUCTION
Antisense imaging was referred to that antisense oligonucleotides of a gene were labeled with radionuclide, then administered to an organism to show its focus, especially the tumor. Colon cancer is a malignant tumor that seriously threatens human health. Its main oncogene, c-myc, whose overexpression can reach 30 times, is a target gene for antisense imaging. The oligonucleotides that are complementary to c-myc mRNA can prohibit many kinds of cancer cells from growing. Many nuclear medicine researchers are interested in this oncogene. At present, the antisense oligonucleotides of c-myc mRNA have been successfully labeled with 99mTc. However, to the author’s knowledge, their application in experimental researches on antisense imaging has not been reported as yet. Is the uptake rate of tumor tissue too small and the background too high to indicate the tumor? How can the uptake rates of tumor cells be increased? Do these limit the application of 99mTc-labeled antisense oligonucleotides? In order to explore the feasibility for antisense imaging of the colon carcinoma, develop a new radiolabeled-gene-pharmaceutics, and promote the progress in the molecular nuclear medicine, the primary experimental studies, antisense imaging, on liposome-entrapped 99mTc-labeled antisense phosphorothioate oligonucleotides as a tracer were carried out.

MATERIALS AND METHODS
Materials
Fifteen mer, single-stranded phosphorothioate oligonucleotides, aminolinked, antisense oligonucleotides targeted at the translation initiation codon of c-myc mRNA were purchased from Gibco-BRL, US. Their base sequences were 5' -NH2 - FACC GTGAGGGG CAT -3' (F stood for phosphorothioate A). The molecular mass of the chain was about 300 u. These oligonucleotides were used directly without further purification, and generally handled under sterile conditions. All solutions were sterilized by terminal filtration through a 0.22 µm filter. All pipettes and tubes were autoclaved prior to use. The oligonucleotides were dissolved at a concentration of 4 mg/mL in sterile water and stored at ~20 °C.

The hydrazino nicotinamide derivatives were synthesized elsewhere. 99 m-pertechnetate was obtained from a 99Mo-99mTc radionuclide generator made by the Chinese Atomic Energy Institute. Tricine, Sncl2·2H2O and Dimethyl sulfoxide were...
supplied by Sigma Company, US, lipofectamin reagent by Gibco-BRL, US, EDTA by Boehringer Mannheim Company, Germany, and Sep-Pak (C18) reverse column by Waters Company, US.

The oligonucleotides were bound to hydrazino nicotinamide derivatives and then labeled with 99mTc following the methods described by Hnatowich et al.10 The 99mTc-labeled oligonucleotides were entrapped with liposome according to the manufacturer’s protocols.

The cellular uptake rates of oligonucleotides

The LS-174-T cells were grown by adherent culture in media (RPMI-1 640, Gibco-BRL, US), supplemented with 100 mL/L fetal bovine serum at 37 °C, 50 mL/L CO2. Thirty-six culture plates with diameter of 33 mm each was inoculated with about 1x10^6 LS-174-T cells and cultured at 37 °C for 48 h. After the cells were grown to about 50% confluence in regular culture media, they were transfected using lipofectamin with 2 μg of freshly prepared liposome-entrapped 99mTc-labeled antisense oligonucleotides with radioactivity of about 29.60 MBq according to the manufacturer’s protocols. The cellular uptake rates were determined at 1, 2, 5 and 7 h, and the testing steps were as follows: The cells were detached by 2.5 g/L trypsin to form suspension, then washed three times with the media by centrifugation (2 500 r/min, 10 min). The supernatant was collected into a 50 mL volumetric cylinder and the precipitation remained in the centrifugation tube. Then the radioactive counts in the precipitation and supernatant were counted in an automatic gamma well counter after correction for decay and background activity separately, and the cellular uptake rates were expressed as a percentage of the total counts. The uptake rate = radioactive counts in precipitation/the total counts in precipitation and supernatant ×100%.

The biodistribution of the antisense oligonucleotides in tumor-bearing nude mice

At first, the tumor model was constructed. Large-scale of LS-174-T cells collected by digestion, centrifugation and washing, were diluted with culture medium without serum and antibiotic to the concentration of 5x10^6 cells per 0.2 mL. Thirty male nude mice, aged about 2 mo, were purchased from Experimental Animal Center of Sichuan University. The mice were maintained in a specific pathogen-free environment and cared in accordance with the institutional guidelines. Each of them was inoculated with 5x10^6 cells at right flank. The tumor was allowed to grow to success. The cellular uptake rates are as follows: 7.21±1.23% at 1 h, 15.19±2.81% at 2 h, 16.13±2.54% at 5 h, and 17.77±2.41% at 7 h. Within 7 h, the cellular uptake rate increased as the time prolonged, and reached the peak at 7 h. It was significantly higher than that at 1 and 2 h. However, the uptake rate at 7 h was not significantly higher than that at 5 h.

The biodistribution of liposome-entrapped 99mTc-labeled antisense oligonucleotides

The biodistribution of liposome-entrapped 99mTc-labeled antisense oligonucleotides are shown in Table 1. The endothelial system played a main role in biodistribution, and accumulated the greater part of the injected dose. The radioactive counts in the tumor tissue increased within 2 h and gradually reached the peak at 2 h, then dropped down sharply.

Table 1

| Tissue   | 0.5 h  | 1 h     | 2 h     | 4 h     |
|----------|--------|---------|---------|---------|
| Liver    | 8.78±0.63 | 9.16±1.14 | 7.92±0.38 | 8.97±0.12 |
| Spleen   | 6.78±0.37  | 8.86±0.60  | 7.37±0.64 | 8.02±0.23  |
| Kidney   | 4.89±0.67  | 2.90±1.19  | 3.07±0.18 | 0.47±0.02  |
| Lung     | 10.2±1.02  | 13.37±0.84  | 12.21±0.42 | 10.2±0.50  |
| Heart    | 7.18±0.13  | 8.78±1.01  | 7.25±0.18 | 5.28±0.45  |
| Blood    | 5.51±0.24  | 4.55±0.15  | 2.81±0.11 | 2.61±0.06  |
| Bone     | 1.84±0.64  | 3.14±1.04  | 1.43±0.24 | 1.02±0.32  |
| Muscle   | 2.27±0.37  | 1.36±0.60  | 1.69±0.91 | 2.85±0.26  |
| Stomach  | 12.45±0.62  | 13.77±0.38 | 10.63±0.35 | 6.70±0.44  |
| Intestines | 4.54±0.42  | 6.68±4.14  | 7.76±0.34 | 8.44±0.63  |
| Brain    | 2.24±0.42  | 2.16±2.10  | 0.68±0.06 | 0.52±0.06  |
| Tumor    | 6.12±0.31  | 8.09±0.86  | 13.46±0.20 | 4.58±0.45  |

P<0.05 vs that of liver tissue P<0.05 vs that of tumor tissue.

Antisense imaging

Anterior imaging at 1 h after intravenous injection of liposome-entrapped 99mTc-labeled antisense oligonucleotides showed that little accumulation of radioactivity might be seen in the right middle flank which was the place of tumor. Anterior imaging demonstrated a little accumulation of radioactivity in tumor site at 1.5 h (Figure 1), and a circular abnormal accumulation focus of radioactivity in the location of tumor at 2 h (Figure 2), but no accumulation of radioactivity in the location of tumor at 24 h.

The ratios of radioactive counts in tumor tissue to that in the contralateral equivalent region

The ratio was the highest one (1.733±0.2537) at 2 h after intravenous injection of liposome-entrapped 99mTc-labeled antisense oligonucleotides. It was significantly higher than...
that at 4 and 6 h \((P<0.05)\). Although it was higher than that at 1.0 and 1.5 h, there was no statistical difference. These ratios are shown in Table 2.

**Table 2** Ratios of the radioactive counts in tumor to that in the contralateral equivalent region of abdomen (mean±SD, \(n=5\) for each time point)

| Time point (h) | Ratios          |
|---------------|----------------|
| 1.0           | 1.2266±1.1259  |
| 1.5           | 1.5597±0.0190  |
| 2.0           | 1.7332±0.2537  |
| 4.0           | 1.0182±0.0495* |
| 6.0           | 1.0199±0.0131* |

\(^*P<0.05\) vs ratio of 2.0 h.

**Figure 1** Anterior imaging at 1.5 h after intravenous injection of liposome-entrapped \(^{99m}\)Tc-labeled antisense oligonucleotides. A little accumulation of radioactivity in tumor site, the right middle flank (arrow) could be observed.

**Figure 2** Anterior imaging at 2 h after intravenous injection of liposome-entrapped \(^{99m}\)Tc-labeled antisense oligonucleotides. A circular abnormal accumulation focus was observed in the location of tumor (arrow).

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