ORIGINAL ARTICLE

High-Dose ^{111}In Induces G1 Cell Cycle Arrest and Cell Death in Rat Bone Marrow Mesenchymal Stem Cells

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Abstract

Purpose This study was performed to evaluate the effect of ^{111}In-labeling on the cell growth, cycle and viability of bone marrow mesenchymal stem cells (BMSCs).

Methods Rat BMSCs were labeled with various doses of ^{111}In (0.4–11.1 Bq/cell). The growth curve of ^{111}In-BMSCs was obtained up to 14th day of labeling. The cell cycle was evaluated by 5-bromo-2-deoxyuridine (BrdU) labeling or propidium iodide (PI) staining. Senescent cells were counted under a light microscope after staining with 5-bromo-4-chloro-3-indolyl-D-galactopyranoside. Flow cytometry was performed to measure apoptotic and necrotic fractions after staining with annexin V-FITC and PI.

Results The growth of BMSCs labeled with higher doses of ^{111}In (4.4 or 11.1 Bq/cell) was significantly inhibited from the 3rd day of labeling. Flow cytometry revealed less BrdU-positive BMSCs at 11.1 Bq ^{111}In/cell during all measurement days and G1 arrest at 4.4 and 11.1 Bq ^{111}In/cell. Significant increases in apoptosis and necrosis were also observed at 4.4 (3.04%/1.35%) and 11.1 Bq ^{111}In/cell (9.07%/3.18%) on the 14th day (control=1.60%/0.39%). However, no cellular senescence was visualized up to the 14th day.

Conclusion A high dose of ^{111}In-labeling induced cell cycle arrest and death in BMSCs; therefore, it should be used with a careful dosimetry in case of applying it to humans.

Keywords ^{111}In-tropolone · Mesenchymal stem cells · Cell cycle · Apoptosis · Necrosis

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Introduction

For more than a decade, investigators have sought an effective method to use stem cells as a regenerative therapy for various diseases. Allogeneic as well as autologous bone marrow mesenchymal stem cells (BMSCs) have been attempted to repair heart or brain diseases, which are supported by the fact that adult stem cells raise few ethical issues as opposed to embryo-derived stem cells [1–3]. In those trials, BMSCs improved left ventricular function in addition to symptoms in patients with myocardial infarction and induced functional recovery in patients with strokes [2, 3].

With the success of cell therapies, a lot of attention has been paid to the monitoring of transplanted stem cells. Non-specific imaging methods providing morphological or functional information such as perfusion or metabolism are

readily available and useful in evaluating the efficacy of cell therapy; however, specific in vivo imaging methods are necessarily required to understand the mechanism and the fate of therapeutic cells [4, 5].

One of the earlier attempts for this purpose is the direct cell labeling with radionuclide [6]. ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO) was used to label several types of bone marrow-derived cells, which were transfused to assess the migration, in vivo distribution and the efficacy of the infusion method in ischemic heart disease [7]. ^{18}F-fluoro-2-deoxy-D-glucose (FDG), which is widely used in clinic for evaluating cancers, cardiac or neurologic disease, was also used to label bone marrow cells in order to monitor in vivo delivery of those cells in patients with myocardial infarction [8]. However, it is generally accepted that ^{111}In is the most suitable radionuclide for tracking cells due to its relatively longer half-life (2.81 days), although positron emitters such as ^{18}F (110 min) or ^{64}Cu (12.7 h) generate higher quality images and ^{99m}Tc (6 h) is cheap and easy to handle. A few researchers have already demonstrated the feasibility of monitoring various therapeutic cells with ^{111}In, where sequential images could be obtained over 2 weeks without killing the animals, by injecting ^{111}In-labeled mesenchymal stem cells, and whole-body images could be acquired in patients receiving CD133+ cell therapy by labeling a fraction cells with ^{111}In [9, 10]. We, recently, also have carried out the study on the migration of rat BMSCs to the traumatically injured brain using ^{111}In-labeling, in which homing of intravenously injected BMSCs to the boundary of the traumatic region was displayed by gamma camera and confocal microscope [11]. However, in the course of that study, we unexpectedly observed that the proliferation of BMSCs was significantly inhibited and the morphology of cells was changed by labeling with a high dose of ^{111}In-tropolone.

In the current study, we therefore investigated further to elucidate the inhibitory effect of ^{111}In-labeling on the growth, cell cycle and the viability of BMSCs.

Materials and Methods

Isolation and Culture of Rat Bone Marrow Mesenchymal Stem Cells

Isolation and culture of rat BMSCs was performed as described previously [11]. Briefly, femurs were removed from adult male Sprague-Dawley rats (Orient Co, Sungnam, Korea) weighing 300 g (8 weeks old) and end-removed bones were centrifuged at 1,800 rpm for 20 min. After centrifugation, cells were rinsed with phosphate buffered saline (PBS; Gibco, USA) and resuspended in low-glucose Dulbecco's modified Eagle's medium (DMEM, low

glucose; Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA). After incubation at 37°C in 5% CO_2 for 10 days, cells were detached by 0.25% trypsin/0.1% EDTA (Sigma, USA), and replated on a 100-mm culture dish in fresh medium. Cells at passage 2 were frozen down and stored in the liquid nitrogen tank until enough cells were collected. We confirmed that isolated cells had the characteristics of BMSCs using anti-CD34, CD105 and Stro-1 antibodies. Rat BMSCs at passage 4 were used in the study.

Labeling of Rat Bone Mesenchymal Stem Cells with ^{111}In-Tropolone

One to two milligrams of tropolone (Sigma, St. Louis, USA) was dissolved in 1 ml normal saline, and then 80 µl tropolone solution was mixed with 37-111 MBq (1-3 mCi) of ^{111}InCl$_3$ (physical half-life=2.83 days, γ-energy=245 and 173 keV, PerkinElmer, Waltham, USA) in 0.05 N HCl. The reaction mixture was incubated for 15 min at room temperature (pH=7.2). Before labeling, the BMSCs were washed with PBS, centrifuged at 1,000 rpm for 3 min, and resuspended in 1 ml PBS. Various doses of ^{111}In-tropolone (0.4, 1.1, 4.4, and 11.1 Bq/cell) were then added to the BMSC suspensions and incubated at room temperature for 20 min. After incubation, BMSCs were centrifuged at 1,000 rpm for 3 min, and supernatants were removed. BMSC pellets were further suspended with PBS or other solutions according to the procedure.

Proliferation and Light Microscopic Examination of ^{111}In-BMSCs

After labeling with four different doses (0.4, 1.1, 4.4 and 11.1 Bq/cell) of ^{111}In-tropolone, 3.0×10^4 BMSCs were seeded in 60-mm culture dishes and grown at 37°C in a 5% CO_2. To obtain growth curves, cells were detached and counted at the 3rd, 6th, 10th and 14th day. For comparison, control BMSCs, which were incubated in PBS without ^{111}In-tropolone, were also grown and counted. On each measurement day, cells were visually examined under a light microscope for morphological changes. The experiment was performed in triplicate for accuracy.

Cell Cycle Analysis

For cell cycle analysis, ^{111}In-BMSCs were labeled with 5-bromo-2-deoxyuridine (BrdU; Sigma) and were stained with propidium iodide (PI) (50 µg/ml; BD Pharmingen, USA) at the 3rd, 6th, 10th, and 14th day. For BrdU labeling, ^{111}In-BMSCs were labeled with BrdU and analyzed using flow cytometer (BD Bioscience, San Jose, USA). A stock solution of 2.5 µl, 10 µM BrdU (in DMSO) was added to

the ^{111}In-BMSCs (0.4, 1.1, 4.4 and 11.1 Bq/cell) in 10 ml sterile culture medium, and then, it was incubate at 37°C in a 5% CO_2 atmosphere for 1 h. Following incubation, cells were washed twice with PBS and re-suspended in ice-cold PBS containing 10% FBS and 1% sodium azide (5×10^6 cells/ml). Cell suspensions (5×10^5 cells/100 μl) and primary antibody (10 μg/ml, abcam, USA) were mixed and incubated in a tube for overnight at 4°C. Cells were washed three times and incubated with 0.5 ml secondary antibody (2 mg/ml, Alexa Fluor 488 goat, anti-rat IgG; Invitrogen, USA) in 3% BSA/PBS for 30 min at 4°C. And then, those cells were washed three times and resuspended in ice-cold PBS with 3% BSA and 1% sodium azide for flow cytometry analysis. For PI staining, BMSCs labeled with four different doses of ^{111}In-tropolone (0.4, 1.1, 4.4 and 11.1 Bq/cell) were divided into three dishes (4.0×10^5 cells), and cells were detached on the 6th day. As a control, unlabeled BMSCs (4.0×10^5 cells) were used. Cells were trypsinized, rinsed twice in 5 ml 0.1% BSA/PBS, fixed with 3 ml cold 70% ethanol, and samples stored in a -20°C freezer until analysis. On the day of analysis, samples were transferred to FACS tubes and resuspended twice with PBS. After removing supernatant, 200 μl of RNase A stock solution (500 μg/ml in PBS; Qiagen, USA) was added to each tube and incubated at 37°C for 30 min. Then, 200 μl PI was added to each tube, incubated on ice for 30 min in the dark and analyzed by FACS with Flowjo software (ver. 7.2.4). The experiment was performed in triplicate.

Cell Senescence Assay

Cell senescence assay was also performed with a commercially available kit (Innoprot, Spain) according to the manufacturer's guidelines. In brief, fixing solution stocks were prepared by diluting a stock solution in PBS (1:100). A total of 100 ml staining solution was made by mixing an 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (X-gal) solution with staining solutions and PBS (50 μl X-gal solution, 10 μl staining solution A, 10 μl staining solution B, 2 μl staining solution C, 200 μl staining solution D and 728 μl PBS). Non-labeled BMSCs (control) and ^{111}In-labeled BMSCs (1.1, 4.4 and 11.1 Bq/cell) were plated on 12-well plates and incubated at 37°C, 5% CO_2 for 3, 6, 10 and 14 days. At each time-point, culture medium including unlabeled ^{111}In-tropolone was removed and cells were fixed by incubating 1 ml fixing solution for 5 min at room temperature. Fixing solution was removed by rinsing the cells with PBS, and then, those cells were incubated with 1 ml staining solution cells at 37°C for 12-24 h, protected from light. Stained cells were rinsed twice with PBS to remove staining solution and examined under a light microscope. Blue-stained cells were determined as senescent cells. The positive control, incubated in a senescent environment, the fasting state, for 7 days was stained with SA-β-galactosidase solution.

Apoptosis and Necrosis Assay

Apoptosis and necrosis assays for ^{111}In-labeled BMSCs were performed with a commercially available kit (BD Pharmingen, USA) and a flow cytometer according to the manufacturer's guideline. BMSCs labeled with four different doses of ^{111}In-tropolone (0.4, 1.1, 4.4 and 11.1 Bq/cell) were divided into three dishes (4.0×10^5 cells) respectively, and grown from the 3rd to 14th day. At each time-point, ^{111}In-labeled BMSCs and control cells were detached with trypsin, and then the cells were washed twice with cold PBS, and resuspended in binding buffer. For each tube, 5 μl annexin V-fluorescein isothiocyanate (FITC) and 5 μl PI were added and the mixture was incubated for 15 min at room temperature in the dark. Within 1 h of incubation, the cells were analyzed by flow cytometer. The experiment was performed in triplicate for accuracy.

Statistical Analysis

Comparison of growth curve, apoptosis/necrosis fraction and G1 cell fraction among subgroups was done using analysis of variance (ANOVA), followed by Student-Newman-Keuls test for pairwise comparison. A p vlaue less than 0.05 was considered to be significant.

Results

Proliferation of ^{111}In-labeled BMSCs

The growth curve and the light microscopic images of ^{111}In-BMSCs are shown in Fig. 1a, b, respectively. ^{111}In-labeling inhibited the proliferation of BMSCs significantly from the 3rd to 14th day in a dose-dependent manner ($p < 0.001$ on the 3rd, 6th and 14th day, and $p = 0.002$ on the 10th day by ANOVA, Fig. 1a). BMSCs labeled with the highest dose (11.1 Bq/cell) of ^{111}In did not proliferate throughout the measurement days. Moreover, those labeled with 4.4 Bq of ^{111}In/cell proliferated slowly and the number of cells was significantly decreased on the 10th and 14th day compared with the controls, which suggested that they could not recover from the effect of ^{111}In-labeling. On the other hand, the number of BMSCs labeled with 0.4 or 1.1 Bq ^{111}In /cell was not significantly different from that of controls except a slight reduction on the 3rd day. Based on the light microscopic images, BMSCs labeled with the lowest concentration of ^{111}In (0.4 Bq/cell) reached confluency on the 14th day, whereas those with the highest dose (11.1 Bq/cell) of ^{111}In did not proliferate (Fig. 1b). Furthermore, those labeled with 11.1 Bq ^{111}In/cell showed morphological changes such a swelling or an increase size.

Fig. 1 **a** The growth curve of BMSCs obtained from day 0 to 14 after labeling with different doses of [111]In (0.4, 1.1, 4.4 and 11.1 Bq/cell). This experiment was performed in triplicate with *error bars* showing standard error; *p* values by ANOVA are marked on each day. **b** Light microscopic images of control and BMSCs labeled with the lowest (0.4 Bq/cell) and the highest doses (11.1 Bq/cell) of [111]In (magnification=×200). Concentrations of ^{111}In were presented as Bq/cell. *Bq* Becquerel; *$p<0.05$ compared with the control
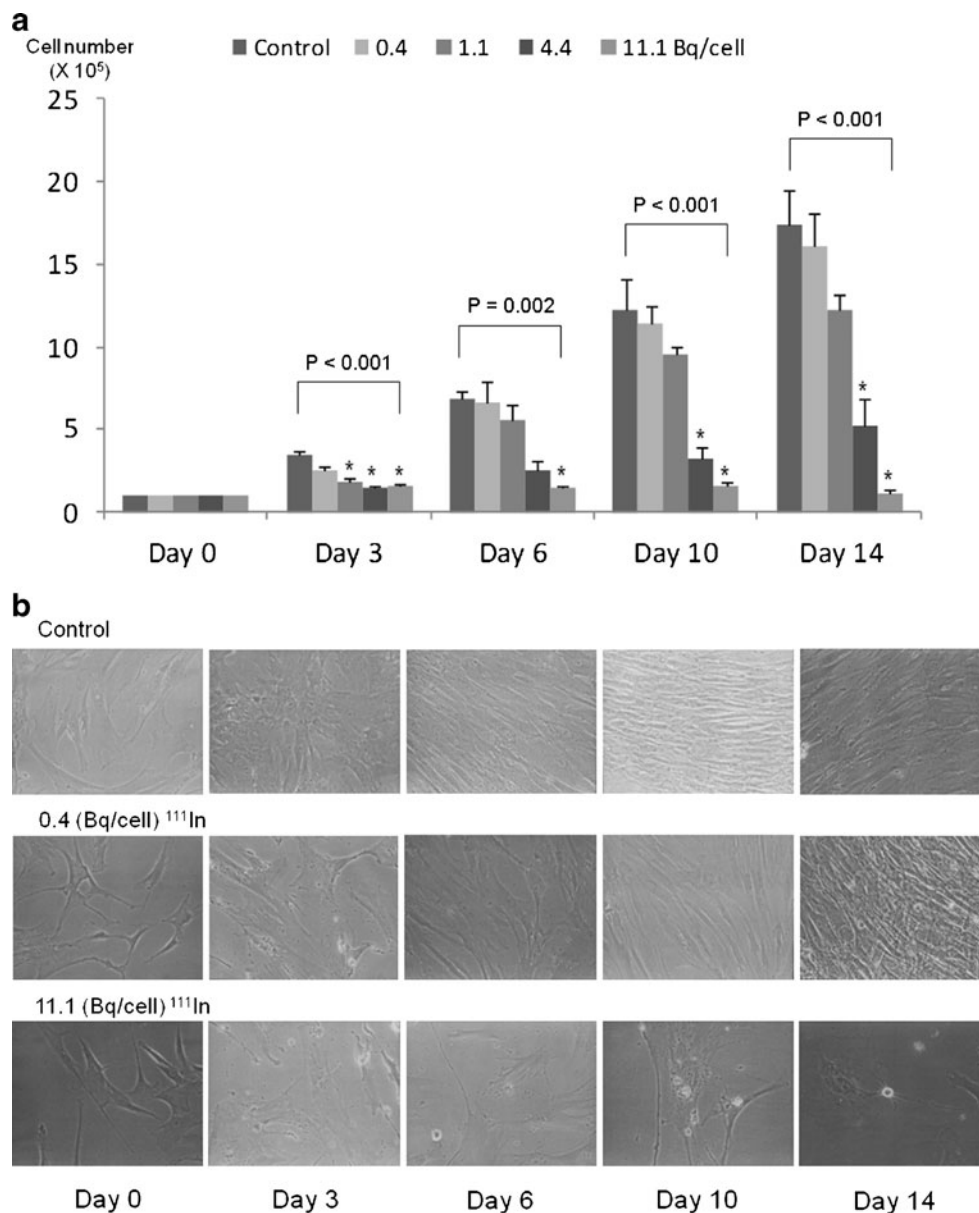
Cell Cycle of ^{111}In-labeled BMSCs

BrdU staining revealed a similar pattern of cell cycle graph in BMSCs labeled with 0.4 Bq/cell of ^{111}In and control (Fig. 2a). However, those with 1.1, 4.4 and 11.1 Bq/cell of ^{111}In showed decreased BrdU(+) fractions on the 3rd day compared with the control. Among these, BMSCs labeled with 4.4 or 11.1 Bq of ^{111}In/cell showed a consistent decrease in BrdU(+) from the 3rd to 14th day, while the pattern of those labeled with 1.1 Bq ^{111}In/cell recovered on the 6th day.

Cell cycle analysis by PI staining revealed G1 arrest in BMSCs labeled with 4.4 or 11.1 Bq of ^{111}In/cell (Fig. 2b). On the 6th day of labeling, G1 fractions of BMSCs labeled with 4.4 and 11.1 Bq of ^{111}In were significantly higher than that of control (48.6% and 53.1% vs 40.0%, all $p<0.05$).

Cellular Senescence of ^{111}In-labeled BMSCs

Under the light microscope, blue-stained, senescent cells were rarely observed at all concentrations (1.1, 4.4 and 11.1 Bq ^{111}In/cell) from the 3rd to 14th day. There were no more than three blue-stained cells in one high power field (×200) of each slide (Fig. 3).

Apoptosis and Necrosis of ^{111}In-labeled BMSCs

Annexin V-FITC/PI staining results of ^{111}In-BMSCs are shown in Fig. 4. Apoptotic and necrotic fractions were rarely seen from the 3rd to 10th day after radiolabeling. However, there was a significant difference in both apoptosis and necrosis among subgroups on the 14th day (Fig. 4) and pairwise

Fig. 2 **a** BMSCs labeled with four different doses of [111]In (0.4, 1.1, 4.4 and 11.1 Bq/cell) were grown for 3, 6, 10 and 14 days, and then were incubated with BrdU-containing media for 1 h. Separation between BrdU(+) and BrdU(-) was done with a BrdU(-) control (not shown). Percentage of BrdU(+) was calculated by the number of BrdU(+) cells divided by total cell count (10,000 cells). **b** BMSCs labeled with the same doses of ^{111}In were grown for 6 days, and then the cell cycle was analyzed by flow cytometer after PI staining; *p* value by ANOVA is marked *above the bars*. Concentrations of ^{111}In were presented as Bq/cell; *$p < 0.05$ compared with the control
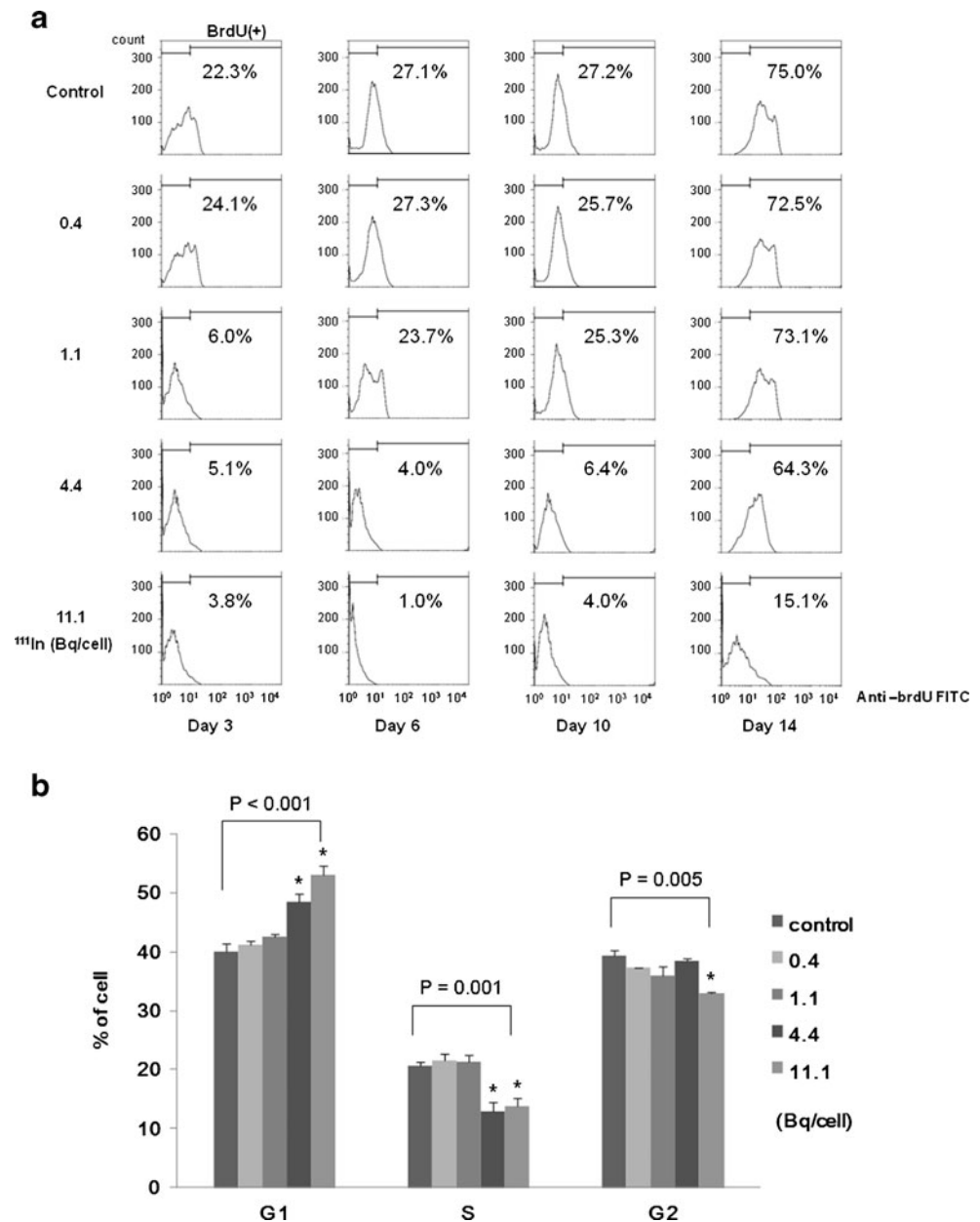
comparison revealed a significant increase in apoptosis/necrosis by 4.4 (3.04%/1.35%) or 11.1 Bq ^{111}In/cell (9.07%/3.18%) compared with the controls (1.60%/0.39%). Even though there was a significant difference in apoptosis and necrosis among subgroups on the 10th day, no significant increase was observed in either apoptosis or necrosis by pairwise comparison.

Discussion

In the present study, we evaluated the effect of ^{111}In-labeling on BMSCs by means of the growth curve, cell cycle and cell death analysis including apoptosis, necrosis and senescence. As a result, ^{111}In-labeling, at high concentrations, inhibited the proliferation of BMSCs and induced G1 cell cycle arrest.

Moreover, ^{111}In-labeling resulted in both apoptotic and necrotic cell death. To our knowledge, this is the first report indicating that cell cycle arrest and cell death were induced in BMSCs by ^{111}In-labeling at slightly higher doses than that used in the clinical cell imaging.

The detrimental effect of ^{111}In-labeling on stem cells has already been reported a few times. ^{111}In-tropolone of more than 0.14 Bq/cell, which is almost the same as our lowest dose in consideration of its labeling efficiency (0.4 Bq/cell, labeling efficiency=35.9%), decreased the viability of BMSCs in a dose-dependent manner [12]. In addition, human mesenchymal stem cells (hMSCs) labeled with 800 Bq ^{111}In/cell did not proliferate as they were adherent, while the doubling time was not influenced within the range 15-260 Bq ^{111}In/cell [13]. According to our results, at 11.1 Bq

Fig. 3 BMSCs labeled with three different doses of ^{111}In (1.1, 4.4 and 11.1 Bq/cell) were grown for 3, 6, 10 and 14 days, and then were stained with SA-β-galactosidase solution for 24 h for the light microscope examination. Blue-stained cells indicate senescent cells. The positive control was grown in a senescent environment (fasting state for 7 days)
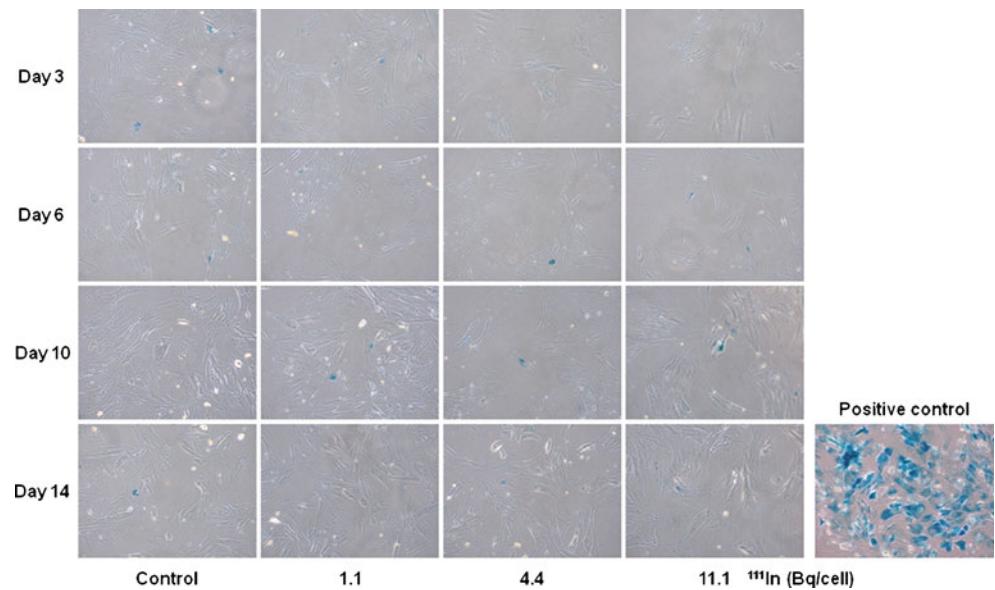
^{111}In/cell there was no significant change in the number of BMSCs throughout the measurement days. This growth inhibition is thought to be caused by cell cycle arrest. When we evaluated the cell cycle, the BrdU(+) fraction was no more than 4% from the 3rd to 10th day, while the G1 fraction was significantly increased on the 6th day (13.1%

higher than control). Furthermore, on the 14th day, there was a significant increase in apoptosis and necrosis, meaning that cell death proceeded in some of those arrested cells after a few days of arrest. On the other hand, at 4.4 Bq ^{111}In/cell, the number of cells gradually increased over time, even though it was significantly smaller than that

Fig. 4 BMSCs labeled with four different doses of ^{111}In (0.4, 1.1, 4.4 and 11.1 Bq/cell) were grown for 3, 6, 10 and 14 days, and then were stained with annexin V-FITC and propidium iodide for apoptosis (**a**) and necrosis (**b**) assay; p values by ANOVA are marked on each day. Percentage indicates the number of apoptotic or necrotic cells divided by total cells; * $p<0.05$ compared with the control
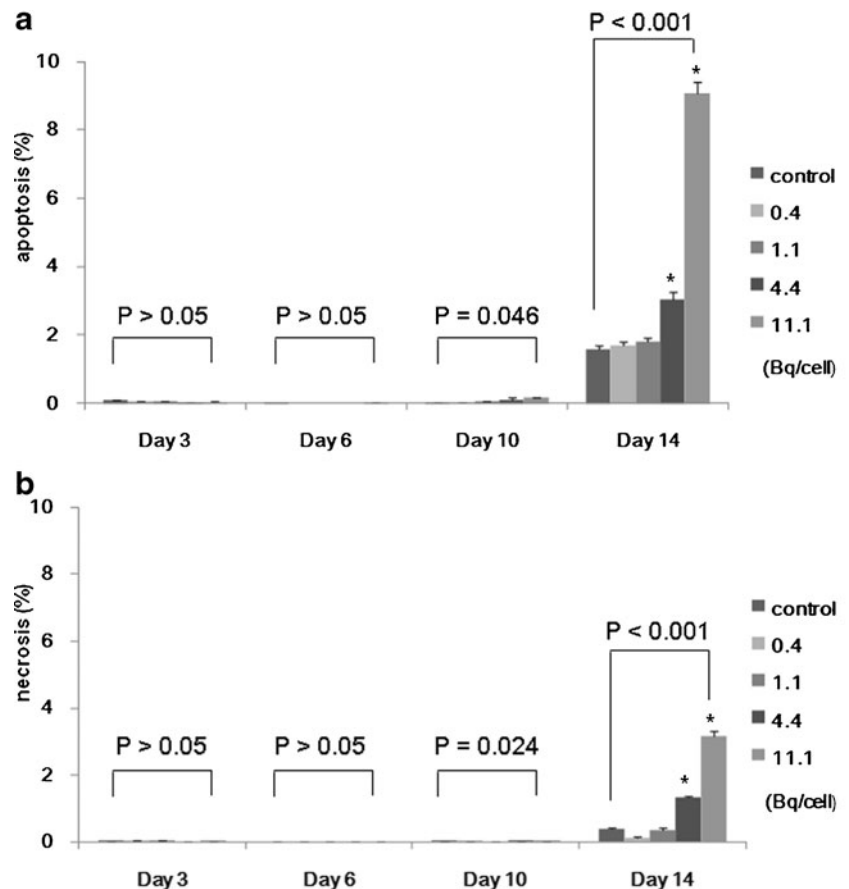
of control on the 3rd, 10th and 14th day. In line with the proliferation, the BrdU(+) fraction was initially very low, from 4.0 to 6.4%, owing to G1 arrest, nevertheless it increased up to 64.3% on the 14th day. Additionally, apoptosis and necrosis on the 14th day were only slightly higher than that of the controls. From these results, it is presumed that at this dose of ^{111}In, a considerable number of arrested cells re-enter the cycle and proliferate when this occurs. Meanwhile, 1.1 Bq ^{111}In/cell showed only a transient growth inhibition and a decrease in BrdU(+) fraction on the 3rd day without leading to a significant cell cycle arrest or cell death. BMSCs labeled with the lowest dose of [111]In (0.4 Bq/cell) did not show any unfavorable change in terms of the proliferation, cell cycle and viability.

Ionizing radiation is known to induce cell cycle arrest and to promote apoptotic cell death in BMSCs. Both G1/S and G2/M arrest were induced by 9-Gy X-ray irradiation, and additionally a 5.2% increase in apoptotic cell death was also observed [14]. On the other hand, γ-ray irradiation (12 Gy, ^{137}Cs) caused G2 arrest (24% increase) with apoptosis (6% increase) and senescence [15]. Unlike those studies, not only apoptosis but also necrosis occurred with ^{111}In at 4.4 or 11.1 Bq/cell, indicating a different level of DNA damage. Furthermore, only G1 arrest was shown and no senescent cells could be seen in this study.

It is very important to consider carefully whether ^{111}In-labeling enhances the proliferation of BMSCs or not in that tumors may develop from BMSCs. It has been reported that tumor having the features of Ewing's sarcoma was generated from the bone-marrow derived mesenchymal progenitor cells [16]. However, in this study, the proliferation of BMSCs was not augmented by ^{111}In-labeling with the concentrations from 0.4 to 11.1 Bq/cell, which indicates that ^{111}In within this range can be used in clinic without worrying about tumorigenesis.

We determined the minimum labeling dose (0.4 Bq/cell) in view of the number of therapeutic BMSCs in stroke patients (10^8 cells) as well as the maximum administered activity for adults (40 MBq) in the clinical leukocyte imaging for infection/inflammation [3, 17]. According to our results, we could use the dose between 0.4 and 1.1 Bq/cell because 0.4 Bq ^{111}In/cell showed no adverse effect on BMSCs, while 1.1 Bq ^{111}In/cell resulted in a transient growth inhibition on the 3rd day without cell death. In consideration of the average labeling efficiency of ^{111}In-BMSCs (35.9%), approximately $1–3 \times 10^8$ cells are required for tracking, which is comparable with the number of hMSCs ($1.3–2.0 \times 10^8$) applied to track therapeutic cells in patients with liver cirrhosis in a recent report [18]. Half of the hMSCs were labeled by ^{111}In-oxine and monitored up to 10 days after infusion without adverse effects within

1 month. Therefore, we could apply ^{111}In-labeling to cell tracking in humans on condition that we adjust the dose carefully.

The limitation of the current study is that we could not estimate the in vivo status of radiolabeled stem cells from in vitro results, because BMSCs may behave differently between in vivo and in vitro. Interestingly, it has been suggested recently that BMSCs could be more protective from the radiation in vivo [19]. Although those results are favorable for the clinical use of ^{111}In-labeling for cell tracking, in vivo the effect of ^{111}In-labeling on BMSCs has to be evaluated further in a follow-up study.

Another limitation of the current study is that we did not compare surface markers before and after ^{111}In-labeling for the characterization of BMSCs nor did we evaluate the differentiation capacity after radiolabeling. We have analyzed surface markers to confirm the isolation of BMSCs before radiolabeling only. In relation to changes in the characteristics of BMSCs after ^{111}In-labeling, a recent study demonstrated that hMSCs labeled with 30 Bq ^{111}In-tropolone/cell (labeling efficiency=25%) and unlabeled control showed identical gene expression after stimulation by vascular endothelial growth factor (VEGF)-A165 and similar surface marker expression (CD73 and CD90). Furthermore, ^{111}In-labeled hMSCs behaved like endothelial cells after VEGF-A165 stimulation [13]. Another recent study supported the fact that ^{111}In-labeling did not affect on the differentiation capacity and characteristics (CD73, CD90 and CD105) of hMSCs [20]. On the other hand, the metabolic activity and migration capacity of hMSCs were impaired by ^{111}In-labeling [20]. Reduced migration capacity by ^{111}In-labeling was also observed in hematopoietic progenitor cells [21]. Taking these reports into consideration, characterization including metabolic activity and migration capacity after radiolabeling is required prior to use in vivo.

In conclusion, a high dose of ^{111}In-labeling could retard cellular proliferation by G1 arrest and might result in apoptotic or necrotic cell death in vitro. Therefore, further in vivo evaluation and a careful dosimetry with characterization of BMSCs are necessary before applying ^{111}In-labeling to patients for the monitoring of therapeutic BMSCs.

Conflicts of Interest None.

References

1. Raval AN, Kamp TJ, Hogle LF. Cellular therapies for heart disease: unveiling the ethical and public policy challenges. J Mol Cell Cardiol. 2008;45(4):593–601.
2. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol. 2004;94(1):92–5.
3. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol. 2005;57(6):874–82.
4. Flores-Ramirez R, Uribe-Longoria A, Rangel-Fuentes MM, Gutierrez-Fajardo P, Salazar-Riojas R, Cervantes-Garcia D, et al. Intracoronary infusion of CD133+ endothelial progenitor cells improves heart function and quality of life in patients with chronic post-infarct heart insufficiency. Cardiovasc Revasc Med. 2010;11(2):72–8.
5. Yang Z, Zhang F, Ma W, Chen B, Zhou F, Xu Z, et al. A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery via a noninfarct-relative artery. Cardiovasc Ther. 2010;28(6):380–5.
6. Bengel FM, Schachinger V, Dimmeler S. Cell-based therapies and imaging in cardiology. Eur J Nucl Med Mol Imaging. 2005;32 Suppl 2:S404–16.
7. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation. 2003;108(7):863–8.
8. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation. 2005;111(17):2198–202.
9. Chin BB, Nakamoto Y, Bulte JW, Pittenger MF, Wahl R, Kraitchman DL. [111]In oxine labelled mesenchymal stem cell SPECT after intravenous administration in myocardial infarction. Nucl Med Commun. 2003;24(11):1149–54.
10. Caveliers V, De Keulenaer G, Everaert H, Van Riet I, Van Camp G, Verheye S, et al. In vivo visualization of ^{111}In labeled CD133+ peripheral blood stem cells after intracoronary administration in patients with chronic ischemic heart disease. Q J Nucl Med Mol Imaging. 2007;51(1):61–6.
11. Yoon JK, Park BN, Shim WY, Shin JY, Lee G, Ahn YH. In vivo tracking of ^{111}In-labeled bone marrow mesenchymal stem cells in acute brain trauma model. Nucl Med Biol. 2010;37(3):381–8.
12. Jin Y, Kong H, Stodilka RZ, Wells RG, Zabel P, Merrifield PA, et al. Determining the minimum number of detectable cardiac-transplanted ^{111}In-tropolone-labelled bone-marrow-derived mesenchymal stem cells by SPECT. Phys Med Biol. 2005;50(19):4445–55.
13. Bindslev L, Haack-Sorensen M, Bisgaard K, Kragh L, Mortensen S, Hesse B, et al. Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation. Eur J Nucl Med Mol Imaging. 2006;33(10):1171–7.
14. Chen MF, Lin CT, Chen WC, Yang CT, Chen CC, Liao SK, et al. The sensitivity of human mesenchymal stem cells to ionizing radiation. Int J Radiat Oncol Biol Phys. 2006;66(1):244–53.
15. Schonmeyr BH, Wong AK, Soares M, Fernandez J, Clavin N, Mehrara BJ. Ionizing radiation of mesenchymal stem cells results in diminution of the precursor pool and limits potential for multi-lineage differentiation. Plast Reconstr Surg. 2008;122(1):64–76.
16. Riggi N, Cironi L, Provero P, Suva ML, Kaloulis K, Garcia-Echeverria C, et al. Development of Ewing's sarcoma from primary bone marrow-derived mesenchymal progenitor cells. Cancer Res. 2005;65(24):11459–68.
17. Palestro CJ, Brown ML, Forstrom LA, McAfee JG, Royal HD, Schauwecker DS et al. Society of Nuclear Medicine Procedure Guideline for ^{111}In-Leukocyte Scintigraphy for Suspected Infection/Inflammation. 2004. http://interactive.snm.org/docs/Leukocyte_v3.pdf
18. Gholamrezanezhad A, Mirpour S, Bagheri M, Mohamadnejad M, Alimoghaddam K, Abdolahzadeh L, et al. In vivo tracking of ([111])In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol. 2011. doi:10.1016/j.nucmedbio.2011.03.008.
19. Singh S, Kloss FR, Brunauer R, Schimke M, Jamnig A, Greiderer-Kleinlercher B, et al. Mesenchymal stem cells show radioresistance in vivo. J Cell Mol Med. 2011. doi:10.1111/j.1582-4934.2011.01383.x.
20. Gildehaus FJ, Haasters F, Drosse I, Wagner E, Zach C, Mutschler W, et al. Impact of indium-111 oxine labelling on viability of human mesenchymal stem cells in vitro, and 3D cell-tracking using SPECT/CT in vivo. Mol Imaging Biol. 2011;13(6):1204–14.
21. Brenner W, Aicher A, Eckey T, Massoudi S, Zuhayra M, Koehl U, et al. ^{111}In-labeled CD34+ hematopoietic progenitor cells in a rat myocardial infarction model. J Nucl Med. 2004;45(3):512–8.