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Serial changes in N-isopropyl-p[^{125}I]-iodoamphetamine in mouse lung observed with a confocal laser scanning microscope

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Serial changes in N-isopropyl-p[^{125}I]-iodoamphetamine (^{125}I-IMP) in mouse lungs were observed with a confocal laser scanning microscope. Male mice were intravenously injected with ^{125}I-IMP and subjected to autoradiographic procedures 20 minutes, and 3 and 24 hours after injection. Differential interference contrast (DIC) images and confocal images were obtained with a confocal laser scanning microscope, and superimposed images were evaluated. Large numbers of silver grains were observed in the interstitium, bronchioles, and alveolar sacs 20 minutes after the injection, and lamellar distribution of the grains was observed on the ciliary surface. The numbers of silver grains in the interstitium and bronchioles had decreased 3 hours after the injection of ^{125}I-IMP, but the numbers of silver grains in the alveolar spaces had not. Although small numbers of silver grains remained in both the bronchioles and alveolar sacs 24 hours after the injection, most of them had washed out.

Confocal laser scanning microscopy is considered to be a useful procedure for studying the distribution of radioisotopes by microautoradiography, because it allows clear autoradiographs to be obtained in which tissues and silver grains are perfectly matched and all silver grains are in focus.

Key words: N-isopropyl-p[^{125}I]-iodoamphetamine, mouse lung, autoradiography, confocal laser scanning microscope

INTRODUCTION

IT IS IMPORTANT and of interest to physicians involved in the practice of nuclear medicine to clarify the serial behavior of radioisotopes and the mechanism of radioisotope accumulation in specific locations *in vivo* but it is very difficult to visually identify the mechanisms of radioisotope accumulation in specific locations at the cellular level.

N-isopropyl-p[^{125}I]-iodoamphetamine (^{125}I-IMP) is clinically used to measure regional cerebral blood flow by single-photon emission computed tomography.[1] It is well known that after being administered, IMP accumulates in the lungs and is washed out with the passage of time.[2] With the confocal laser microscope, very clear and precise microautoradiographs can be obtained compared with the conventional light microscope, as has been reported by Watanabe et al.[14] The present study was undertaken to determine the exact location of IMP in the lungs at certain intervals after intravenous injection of IMP by means of autoradiography, and to ascertain the usefulness of the confocal laser scanning microscopy for observation of the autoradiographs.

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MATERIALS AND METHODS

Animals. Twenty-six adult male ICR strain mice (Nihon Clea Inc., Osaka, Japan) weighing 25–27 g each were used. The mice were individually caged in a temperature-controlled room (23°C) and were given access to water and a standard diet (CE-2, Clea Japan, Osaka, Japan) ad libitum. A standard illumination schedule of 12 hours in the dark, with lights on at 0600 hours, was used. This experimental protocol was approved by the Ethics

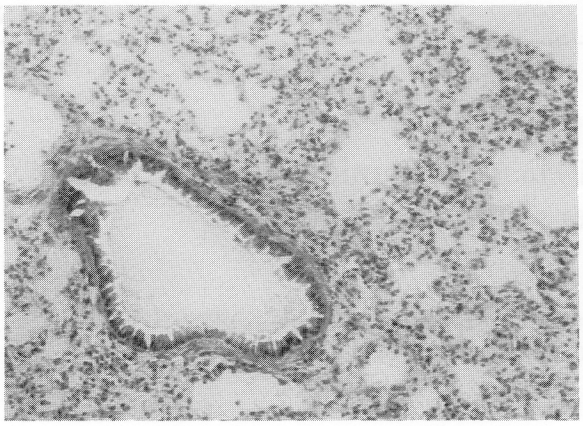

Fig. 1 A differential interference contrast (DIC) image obtained 20 minutes after i.v. injection of ^{125}I-IMP. The histological features of the bronchioles and alveoli are satisfactorily visualized (hematoxylin staining).

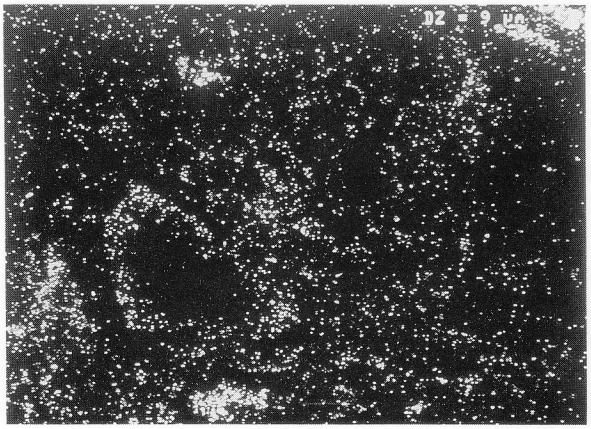

Fig. 2 A confocal image of reflectance from silver grains developed in the autoradiograph.

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Labeled compound. ^{125}I-IMP (specific activity 44.4 kBq/μg, Nihon Medi-Physics, Nishinomiya, Hyogo, Japan) was dissolved in Ringer's solution to a concentration of 925 kBq/m*l*.

In vivo *microscopic autoradiography.* The mice were intravenously (i.v.) injected with 370 kBq ^{125}I-IMP, and were killed by cervical dislocation 20 minutes and 3 and 24 hours after the injection. Their lungs were removed and frozen in liquid N_2, and cryosections 20 μm thick were cut with a cryomicrotome (CM 3050, Leica Instruments GmbH, Mussoloch, Germany). Cryosections were freeze-dried overnight at –20°C in the cryochamber, and subjected to dry-mounting autoradiography. In dry-mounting autoradiography, a gel emulsion film in a wire loop was applied to the section on a glass slide. The wire loop was dipped in melted emulsion containing 0.04% dioctyl sodium sulfosuccinate to prepare the film (Nagata, 1992). In the present study, Konica NR-M2 nuclear emulsion (Konica Photo Co., Tokyo, Japan) was used, and the slides were exposed to the emulsion in a dark box at 4°C for 2 weeks. The emulsion was developed in Kodak D-19 diluted with an equal volume of distilled water at 20°C for 2 min, and the tissue was fixed in 30% sodium thiosulfate solution and stained with hematoxylin.

Laser scanning microscopy. We examined the specimens with a confocal laser scanning microscope (LSM) (LSM-10; Carl Zeiss, Oberkochen, Germany) equipped with a 488 nm argon laser. The autoradiographs were examined with the laser-scanning differential interference contrast (DIC) mode in transmitted light or the confocal LSM mode. The silver grains located at various depths were detected as reflectance, and the LSM images were stored on a hard disk for subsequent display and analysis. Certain confocal LSM images of the reflectance from developed silver grains coincided with the images obtained in the DIC mode. Photographs were taken with a color image recorder (CIR-310, Nippon Avionics Co., Ltd., Tokyo, Japan) equipped with a 35-mm camera.

RESULTS

The results described below were obtained in mouse lungs removed 20 minutes after i.v. injection of ^{125}I-IMP

DIC images were acquired by permeating tissue specimens with a laser beam, and satisfactory histological findings were obtained from the DIC images (Fig. 1). Confocal images were acquired when the laser beam was reflected by silver grains. All silver grains in the confocal images were in focus (Fig. 2). Superimposed DIC and confocal images were prepared, in which tissues and silver grains were in focus and coincided perfectly (Fig. 3).

The changes in IMP over time were as follows

^{125}I-IMP was widely distributed in the interstitium, lumina and epithelia of the bronchioles and alveolar sacs 20 minutes after the injection (Figs. 3 and 4), and lamellar distribution of ^{125}I-IMP was observed on the ciliary surface of the lumina of the bronchioles. Distribution of ^{125}I-IMP was also observed in the basal region of the cylindrical cells (Fig. 3). The amount of ^{125}I-IMP in the interstitium and bronchioles decreased, but ^{125}I-IMP persisted in the alveolar sacs (Fig. 5). Small amounts of ^{125}I-IMP remained in the alveolar sacs and bronchioles 24 hours after the injection, but most of it had been washed out of the lungs (Fig. 6).

DISCUSSION

It is well known that IMP accumulates in the lungs and washes out with the passage of time. It has been reported that 96% of IMP is trapped when IMP reaches the lungs in the first pass.[3] Effros et al. reported detecting i.v.

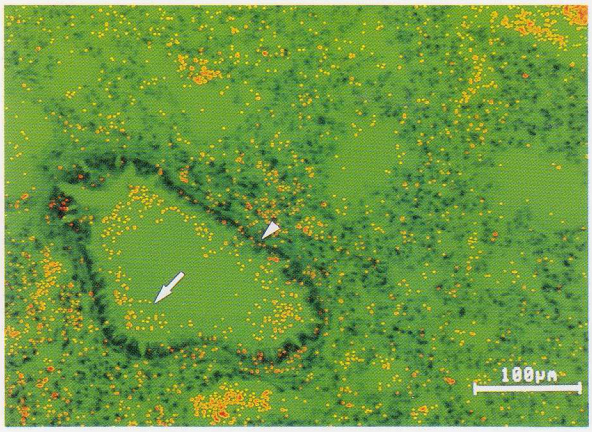

Fig. 3 A superinposed DIC image and confocal image. The silver grains are stained yellow, and regions showing especially strong reflection are stained red. Silver grains were detected in the lumina of bronchioles and interstitium. In the wall of bronchioles, silver grains are seen on the ciliary surface (arrow) and at the basal region of columnar epithelial cells (arrowhead). The bar represents 100 μm.

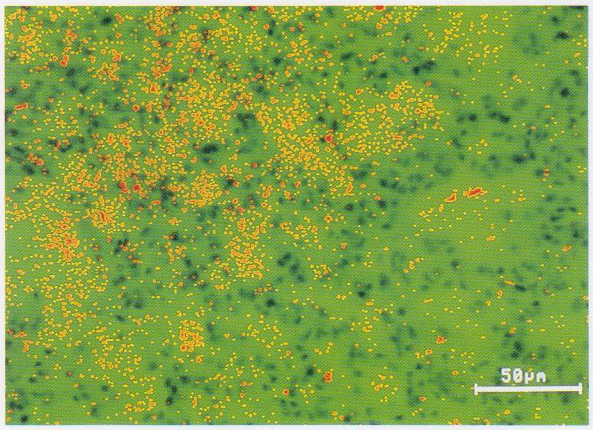

Fig. 4 Image 20 minutes after i.v. injection of ^{125}I-IMP. Silver grains are also seen in the alveolar sacs. The bar represents 50 μm.

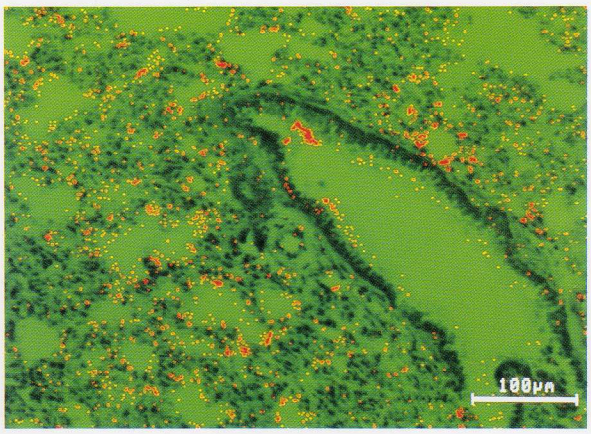

Fig. 5 Image 3 hours after i.v. injection of ^{125}I-IMP. The ^{125}I-IMP in the interstitium and lumina of the bronchioles has decreased, but it is still present in the alveolar space. The bar represents 100 μm.

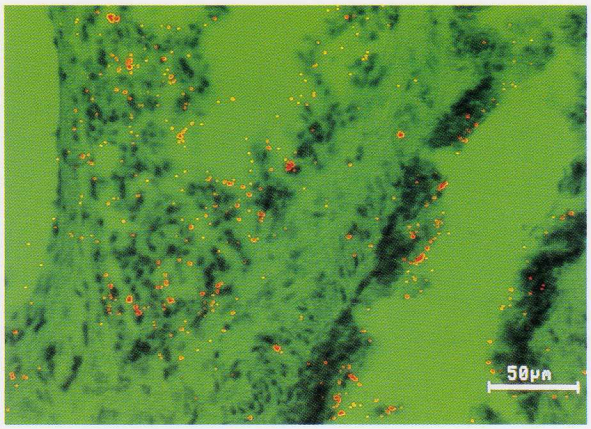

Fig. 6 Image 24 hours after i.v. injection of ^{125}I-IMP. Although small amounts of ^{125}I-IMP remain in the alveolar sacs and bronchiolar lumina, but most of it has washed out of the lungs. The bar represents 50 μm.

injected urea in bronchoalveolar lavage fluid (BALF) 2 minutes after the injection,[4] but there have been no reports describing the membrane permeability of relatively large molecules, such as IMP, or the clearance of the IMP bound to amine receptors.

Examination of serial changes in injected ^{125}I-IMP revealed migration of ^{125}I-IMP from blood vessels into the alveolar sacs and lumina of the bronchioles within 20 min after injection. There are three possible routes by which the radioactivity reaches the lumen of the bronchioles. The first is the route via the alveolar sacs into which ^{125}I-IMP passes through the alveolar walls, which are mainly composed of very flat squamous epithelial cells. The second is a more direct route via the bronchiolar wall, which is composed of a single layer of columnar epithelial cells, because the radioactivity in the bronchioles was observed on the ciliary surface of the columnar epithelial cells and in the basal region, in addition to the columnar cells themselves. The third is a combination of these two routes. The physiological function of the transport of ^{125}I-IMP and/or its metabolites is obscure, but Kato et al. reported that IMP clearance is delayed in the lungs of smokers.[5] The amount of IMP remaining in the lungs of patients with pulmonary diseases 24 hours after i.v. injection has been reported to differ from the amount remaining in normal lungs,[6] and differences have been reported in the amount of IMP in irradiated regions and unirradiated regions.[7] Ronald et al. reported that 20% of IMP is converted to p-iodoamphetamine (PIA) within 2 hours after injection, and the remaining 80% does not metabolize.[8] In the present study, it remained unclear how much of the radioactive IMP that migrated into the respiratory

tract was converted to PIA. The data obtained by microautoradiography may imply that IMP is cleared from the lungs not only by back diffusion into blood vessels, but also by migration of IMP to the respiratory tract shortly after injection. In attempts to explain the mechanism of IMP accumulation in the lungs, various competitive experiments have demonstrated that IMP is bound to amine receptors in the lungs.[2,9–11] Tanaka et al. reported that IMP is bound to amine receptors at 2 binding sites, and they also reported various constants for the respective binding sites.[12] These findings suggests that the mechanism of IMP transported is via receptor-mediated transport pathways, although further experiments are needed to elucidate the mechanism of these substrates.

In the present study, we observed the serial changes in ^{125}I-IMP in mouse lungs by microautoradiography with a confocal laser scanning microscope. Because confocal laser scanning microscopy facilitates observation of the distribution of fluorescent antibodies, it has been reported to be useful to reveal distinct positive staining of thyroid gland cancer.[13] Watanabe et al. reported that images in which both tissue and silver grains were in focus were obtained by confocal laser scanning microscopy, and that all silver grains contained in an emulsifying agent could be visualized by this procedure[14] so that very clear and precise microautoradiographs can be obtained by using this procedure.[15] In conclusion, this study demonstrated the usefulness of microscopic autoradiography in combination with confocal laser scanning microscopy for basic studies in nuclear medicine.

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