A Detailed Examination of Pulmonary Uptake of \textsuperscript{99m}Tc-Tin Colloid in Healthy Mature Miniature Pigs

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Abstract. \textsuperscript{99m}Tc-Tin colloid is a commonly used colloidal radiopharmaceutical in human medicine for evaluating liver function and morphology. \textsuperscript{99m}Tc-Tin colloid is taken up in the liver by the phagocytic activity of Kupffer cells, the reticuloendothelial cells of the liver. Unlike what occurs in human beings, we demonstrated \textsuperscript{99m}Tc-Tin colloid uptake within the lungs and liver in healthy, mature, miniature pigs. Our observations may be explained by the presence of pulmonary intravascular macrophages (PIMs) closely apposed to the endothelium of the pulmonary capillaries in several animal species, such as the sheep, horse, cow, goat, deer, cat and pig. In the current study, we compared scintigraphic images using \textsuperscript{99m}Tc-Tin colloid in rats with those in mature, miniature pigs, and identified the presence of PIMs, reticuloendothelial cells similar to Kupffer cells, by immunohistochemistry in pigs. Pulmonary uptake of \textsuperscript{99m}Tc-Tin colloid occurred only in pigs, and PIMs in the pulmonary capillaries stained positively for mouse monoclonal MAC387 antibodies to macrophages in lung sections, as well as Kupffer cells in liver sections. Therefore, we conclude that the uptake of intravenously injected \textsuperscript{99m}Tc-Tin colloid within both Kupffer cells and PIMs results in scintigraphic imaging of the lung and liver in miniature pigs.

Scintigraphic liver imaging is performed after injection of a \textsuperscript{99m}Tc colloid that has been shown to be rapidly phagocytized by the reticuloendothelial cells of the spleen and bone marrow, as well as by Kupffer cells in the liver (1). Liver imaging can be used for determining the size and shape of the liver and detecting functional abnormalities of the reticuloendothelial cells within the liver (2). Specifically, the current study was performed for suspected focal nodular hyperplasia of the liver and to assess the function of the reticuloendothelial system (RES) in patients with suspected liver disease (1).

Pulmonary intravascular macrophages (PIMs) are large mononuclear cells junctionally adherent to the endothelium of the pulmonary capillaries and morphologically similar to hepatic Kupffer cells (3). PIMs occur in the sheep, horse, cow, goat, deer, cat and pig; in these species, PIMs act as a part of the RES in clearing particles such as bacteria, cellular debris, fibrin, colloids, and liposomes from circulating blood (3, 4). However, in humans and other species, such as the dog, rodents and monkeys, it is generally accepted that the lung does not contain PIMs under normal conditions (3-6).

Porcine liver transplantation is superior to that from other animal sources because of anatomic similarities between pigs and humans (7, 8). However, when hepatoscintigraphic evaluation is performed after liver transplantation, lung uptake of colloid is detected in pigs because of the presence of PIMs.

The purpose of our study was to compare scintigraphic images using \textsuperscript{99m}Tc-Tin colloid in rats and mature miniature pigs, and to identify the presence of PIMs and reticuloendothelial cells such as Kupffer cells, by immunohistochemistry in pigs.

Materials and Methods

Animals. The care, maintenance and treatment of animals in this study were carried out in accordance with the Guidelines for Animal Experiments of the Chonnam National University. Four 18-month-old male healthy, miniature pigs weighing 30-35 kg were purchased from PWG Genetics, Korea. The pigs were caged in single-unit housing under the same conditions and fed dry food twice daily, with water provided \textit{ad libitum}. Pigs were allowed to adjust to their local housing conditions and to the investigators over a period of 7 days. The daily rhythm was maintained by making use of alternating dark and light cycles of 12 h duration each.

Scintigraphic imaging with \textsuperscript{99m}Tc-Tin colloid in miniature pigs and \textit{rats}. Pigs were premedicated with atropine sulfate (0.05 mg/kg), and anesthetized with a combination of xylazine (2.2 mg/kg) and tiletamine/zolazepam (6 mg/kg) by intramuscular injection. Anesthesia was maintained using intravenous propofol at 0.2
mg/kg/h. The pigs were positioned in dorsoventral recumbency, and injected intravenously via the ear vein with 5 mCi of $^{99m}$Tc-Tin colloid. Twenty minutes after injection, dorsoventral, ventrodorsal, left lateral and right lateral static images were acquired using a dual-headed variable angle gamma camera (GE Infinia™ Hawkeye®, GE Healthcare, Hayes, UK). Data were recorded on a 256x256 matrix, with a 20% window centered around the 140-keV photopeak.

Rats were premedicated with atropine sulfate (0.1 mg/kg subcutaneously) and anesthetized with a combination of xylazine (10 mg/kg) and ketamine (40 mg/kg) intraperitoneally. The rats were positioned in dorsoventral recumbency, injected intravenously via the caudal vein with 0.5 mCi of $^{99m}$Tc-Tin colloid, and the acquisition of scintigraphic images was identical to those for the pigs.

**Immunohistochemistry of lung and liver sections from miniature pigs.** Histological examination was performed on the liver and lung of the pigs at the end of the study to identify the presence of PIMs within the lung capillaries. The pigs were sacrificed using an overdose of xylazine, tiletamine/zolazepam, and KCl. The lung and liver were removed and fixed in 10% neutral formalin. All the specimens were embedded in paraffin and 5 μm axial sections were obtained. Sections were deparaffinised in xylene and rehydrated in a graded alcohol series. Immunohistochemical analysis was carried out using the commercially available mouse monoclonal antibody against macrophages (MAC387; Abcam, Cambridge, MA, USA).

**Results**

**Scintigraphic imaging with $^{99m}$Tc-Tin colloid in miniature pigs and rats.** Twenty minutes after the intravenous administration of $^{99m}$Tc-Tin colloid, dorsoventral, ventrodorsal, left lateral, and right lateral static images were acquired using a dual-headed variable angle gamma camera. In the pig, we could not assign morphological features to the liver because the uptake of radiolabeled colloids occurred in both the lung and liver (Figure 1A). In contrast, there was only liver uptake in the rat, thus the morphological features of the liver were clearly seen (Figure 1B).

**Immunohistochemistry of lung and liver sections from miniature pigs.** Immunohistochemical analyses were performed on porcine liver and lung using mouse monoclonal MAC387 antibodies. Intravascular mononuclear cells within the lung capillaries and Kupffer cells in the liver stained for MAC387 antibodies (Figure 2).

**Discussion**

During hepatoscintigraphy, radioactive colloids, such as $^{99m}$Tc-Tin and $^{99m}$Tc-sulfur, are phagocytized by the reticuloendothelial cells of the liver, spleen, and bone marrow. The distribution of the colloids can be imaged by a gamma camera. Intravenously administered $^{99m}$Tc-Tin colloid can facilitate imaging the function of the RES of the liver, and therefore $^{99m}$Tc-Tin colloid can be used to evaluate various hepatic disorders, such as cirrhosis, hepatitis, and metastatic disease (1, 2). In addition, $^{99m}$Tc-Tin colloid can be used as a useful diagnostic tool pre- and postliver transplantation because of its ability to evaluate hepatic function (9).

Particles flowing in the blood stream, such as aged red blood cells, bacteria, endotoxins, cellular debris, and immune complexes, are rapidly removed from blood by macrophages. This system was classically referred to as the RES and is now termed the mononuclear phagocyte system (MPS); these macrophages are mononuclear cells derived from the bone in vivo 23: 551-554 (2009)
marrow. The macrophages enter the circulation and localize in various organs, where they mature into tissue macrophages and play an important role in host defenses. Macrophages with direct access to the circulating blood have historically been reported in the liver, spleen and bone marrow (5). However, in several animal species, such as the sheep, horse, cow, goat, deer, cat and pig, the lung is also a significant site for intravascular macrophages (10, 11). These PIMs are large (20-80 µm in diameter), mature macrophages that are bound to the pulmonary capillary endothelium. PIMs have characteristic morphological features of differentiated macrophages, such as an irregular shape, an indented nucleus, lysosomal granules, pseudopods, phagosomes, phagolysosomes, tubular micro-pinocytosis vermiciformis structures and a fuzzy glycocalyx (12, 13). Therefore, in these animals, pulmonary uptake of radioactive colloids occurs normally, as well as in the liver, and in our studies, the uptake of 99mTc-Tin colloid occurred in the lung and liver in mature miniature pigs. Occasionally, increased radiocolloid uptake is observed in the lung or kidneys; this effect is usually related to the patient’s disease, rather than to such technical factors as macroaggregation of the radiopharmaceutical before injection (14). Reported factors associated with pulmonary uptake of radiocolloids include bacterial endotoxin, malignant lymphoma, intra-abdominal abscesses, advanced breast carcinoma, hepatocellular disease, hepatic failure, systemic amyloidosis and neoplasia (15-17). Some studies in animals and humans have suggested that deposition of fibrin and fibrin degradation products in the lungs could be a potential cause for the pulmonary accumulation of radiocolloids (18). However, considering that the pigs used in our study were healthy, the lung uptake observed here may be explained by the presence of PIMs, rather than the aforementioned factors.

In the current study, we compared scintigraphic images using 99mTc-Tin colloid in rats and mature miniature pigs, and identified the presence of PIMs, reticuloendothelial cells like Kupffer cells, by immunohistochemistry in pigs. As a result, 99mTc-Tin colloids were trapped, not only by Kupffer cells in the liver, but also by PIMs in the lung in pigs. Our results confirmed the presence of PIMs in mature miniature pigs, suggesting that radioactive colloids are not suitable for evaluating liver size, shape, and function because of their non-specific affinity in pigs. Therefore, it is necessary in the pig to establish a method for evaluating hepatic function, such as imaging agents for asialoglycoprotein receptor (ASGPR), which resides exclusively on the plasma membrane of functioning mammalian hepatocytes (19, 20).

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References