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Research article

In vivo uptake and localization of ^{99m}Tc-pertechnetate in pigs using single-photon emission computed tomography

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Abstract

Radionuclide scintigraphy in animals provides valuable information about pathologic and physiologic processes. Technetium-99m (^{99m}Tc) has played an important role in diagnostic imaging over the last several decades. It can be used in the form of either ^{99m}Tc-pertechnetate or ^{99m}Tc-labeled radiopharmaceuticals. The *in vivo* uptake and localization of ^{99m}Tc-pertechnetate in pigs were studied using single-photon emission computed tomography (SPECT). The ^{99m}Tc-pertechnetate was administered to pigs by intravenous and intragastric injections to compare their uptake and localization. The effect of anesthesia in pigs on ^{99m}Tc biodistribution was observed through imaging. The results showed that unconsciousness in the pig induced by isoflurane resulted in the irregular uptake of ^{99m}Tc-pertechnetate when it was directly administered intragastrically. This behavior might have been due to isoflurane reducing the pig's gastrointestinal motility. Data on the nuclear medicine tomographic imaging technique using gamma rays in live animals are limited. Therefore, this observation provided useful information for basic biological research on animal models using SPECT. It is also benefit to the development of new ^{99m}Tc-labeled radiopharmaceuticals in diagnostic nuclear medicine.

Introduction

Radionuclide scintigraphy has a major role in screening for a variety of abnormalities in animals and humans as it provides very useful information about the functional anatomy of an organ when a specific labelled compound is used (Bernier et al., 1997). Technetium-99m is the most commonly used radionuclide in the field of nuclear imaging and is involved in 80% of scintigraphies due to its many advantages, including it decays to technetium-99 with a half-life of 6.02 hr and emits a gamma photon at 140 keV, which falls within the optimal range of gamma cameras (International Atomic Energy Agency, 2009). Furthermore, it can be tagged or attached to a variety of chemicals so that it can be used for the diagnosis of various parts of the body (Smith, 1964). Single-photon emission computed tomography (SPECT), when used with ^{99m}Tc, can provide three-dimensional (3D) images of a region of interest (Bernier et al., 1997). Therefore, it can be used to provide information about localized function in internal organs.

Swine have been one of the major animal species used for research and pharmaceutical development applications because of their similarity to humans in terms of the anatomy and physiology of the gastrointestinal tract (Fleming and Arce, 1986; Gregory et al.,

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1990; Kararli, 1995). Furthermore, the bacterial flora of the colon and the digestion characteristics of small intestines are similar (Rowan et al., 1994). The circulatory system of the pig consisting of heart and blood vessels is similar to the cardiovascular system in humans (Bode et al., 2010). In addition, the kidneys of pigs have many similarities with those of humans in terms of anatomy, function, size and structure (Swindle and Smith, 1998).

The development of a pig model for radionuclide scintigraphy plays a major important role in both pharmacology and toxicology studies as well as in pharmacokinetic study. Few studies and little research exist regarding 3D imaging in pigs using ^{99m}Tc. The purpose of this study was to investigate the *in vivo* uptake and localization of ^{99m}Tc in pigs by using SPECT. Scintigraphic images obtained by intravenous (IV) and intragastric (IG) administrations in conscious and unconscious conditions were investigated to observe whether those parameters affected the bio-distribution pattern of ^{99m}Tc-pertechnetate.

Materials and Methods

Animals

Two healthy female pigs weighing (15–25 kg) were selected and housed in individual coops under standard environmental conditions at an ambient temperature of 25°C. The pigs were allowed 1 wk to acclimatize prior to the commencement of the study. Water and feed were offered *ad libitum*. The animals were fasted overnight before starting the experiment.

The animal experiments were carried out in accordance with the guidelines of the 'Ethical Principles for the Use of Animals for Scientific Purposes' issued by the National Research Council of Thailand (NRCT) and approved by the Animal Experiment Committee of Kasetsart University (Approval no. AC KU 60−Vet−012).

Materials

The sodium pertechnetate ($Na^{99m}TcO_4$) solution was obtained from Thailand Institute of Nuclear Technology (Public Organization), Thailand. It was eluted from a ^{99}Mo/^{99m}Tc generator (ELUMATIC III, CIS bio international, France). The combination of anesthetic agent, tiletamine HCl and zolazepam HCl, (Zoletil® 100) was purchased from Virbac, UK. Isoflurane was obtained from Troikaa Pharmaceuticals Ltd., India.

Imaging Study

This study investigated the behavior of sodium pertechnetate bio-distribution pattern in swine between unconscious and conscious cases via IV and IG administrations. Descriptive and exploratory analyses were conducted to examine the localization and fate of the ^{99m}Tc-pertechnetate. Image acquisition was carried out using a symbia SPECT (Siemens Healthcare, USA) at the Veterinary Teaching Hospital, Kasetsart University. The scintigraphy images were visually compared qualitatively.

In the unconscious pig study, the animal received premedication with Zoletil® 100 (5 mg/kg BW) prior to general anesthesia. The animal was intubated using an endotracheal tube for inhalation anesthesia. Then, the pig was pre-oxygenated (oxygen flow 3−5 L/min) for 3−5 min and isoflurane was gradually induced up to 5%. Anesthesia was maintained with end-tidal isoflurane 3% and an oxygen-air mixture (fraction of inspired oxygen, 0.6) via mechanical ventilation.

In contrast, the study of the radiotracer bio-distribution pattern in a conscious pig was performed using only a tranquilizer (Zoletil® 100) before and during the experiment. The premedication of Zoletil® 100 (5 mg/kg bodyweight, BW) was intramuscularly injected into the animal. After ^{99m}Tc uptake via either the IV or IG route, the pig was allowed to regain consciousness. Subsequently, anesthesia was induced with Zoletil® 100 (10 mg/kg BW) via an IV injection 5 min before starting the SPECT imaging. Thereafter, the pig was allowed to regain consciousness and to rest in a cage for approximately 50 min before the next imaging. The protocol was performed for three cycles of the scan.

For the conscious and unconscious studies, scintigraphic images between IV and IG administrations were investigated. For the IV study, the pig was injected directly into the ear vein with 5 mCi/10 mL sodium pertechnetate through an IV catheter. SPECT images were acquired at 1 hr, 2 hr, 3 hr and 4 hr after IV injection of the sodium pertechnetate.

To compare with the IV study, the same pigs were used after being allowed to rest for 7 d to wash out any radiotracer prior to performing the IG study.

For direct IG injection, ultrasound was used to locate the area of the stomach. Following the location test and identification of the cavity by the injection of 5 mL normal saline solution (NSS), 5 mCi/10 mL sodium pertechnetate was introduced directly into the cavity of the stomach and the syringe was flushed with 40 mL NSS. In the preliminary result, the scintigraphy scans during the first hour showed no alteration in the stomach. Therefore, SPECT imaging following the IG route was continued at 2 hr, 3 hr and 4 hr. The overall experiment is summarized in Fig. 1.

Results and Discussion

Localizations of ^{99m}Tc-pertechnetate in pigs using SPECT are shown in Figs. 2−5. The fate and distribution of ^{99m}Tc-pertechnetate in unconscious (Figs. 2 and 3) and conscious pigs (Figs. 4 and 5, respectively) using SPECT are presented.

Comparison views of the ^{99m}Tc distribution at 1 hr, 2 hr, 3 hr and 4 hr following IV injection in the unconscious pigs obtained by coronal, sagittal and transaxial images are demonstrated in Fig. 2. At the end of the first hour, the ^{99m}Tc activities were predominantly located in the gastric mucosa. Low activity of gamma ray was presented in the urinary bladder. In contrast, ^{99m}Tc mostly accumulated higher in the urinary bladder than in the gastric mucosa at the second hour of administration. Thereafter, the elimination phase of radiotracer showed an increasingly extent of ^{99m}Tc in the bladder following the third and fourth hours, with a certain amount of ^{99m}Tc remaining in the gastric mucosa. It was confirmed that the fate and distribution of ^{99m}Tc-sodium pertechnetate following IV injection was regularly taken up and excreted in the inhaled unconscious pig.

The bio-distribution pattern of IV injection in unconscious pigs in this study coincided with earlier data reported in a case of a boy aged 21 mth with recurrent gastrointestinal bleeding. Technetium-99m scintigraphy showed extensive tracer accumulation throughout the jejunum and proximal ileum (Heinrichs et al., 1997). The results confirmed the advantages of sodium pertechnetate scintigraphy in detecting ectopic gastric mucosa as a possible source of gastrointestinal bleeding.

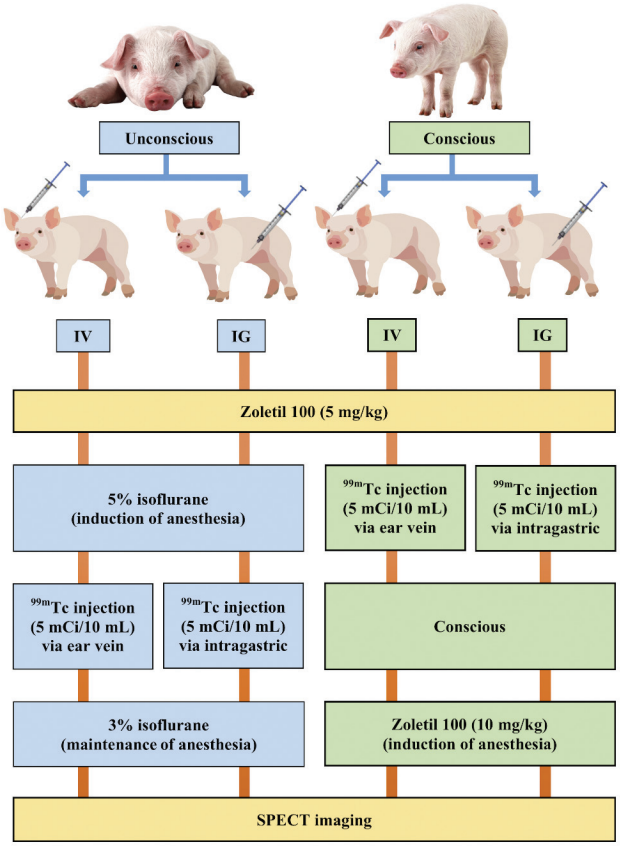

Fig. 1 Diagram of the overall experiment

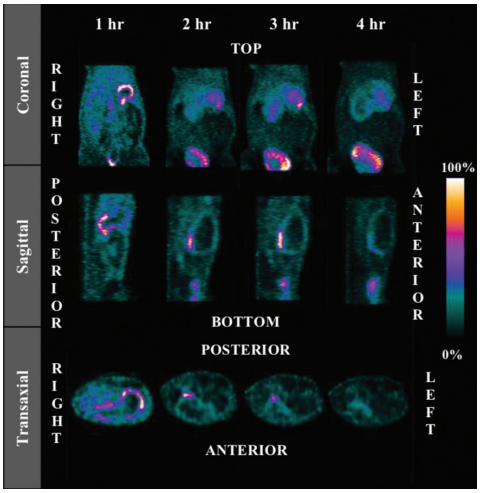

Fig. 2 Coronal, sagittal and transaxial images of unconscious pig obtained using SPECT scanner at 1 hr, 2 hr, 3 hr and 4 hr after intravenous injection of ^{99m}Tc-pertechnetate, where color intensity is proportional to pixel activity (percentage)

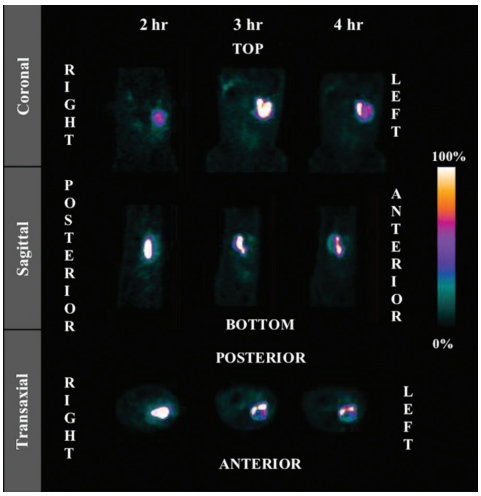

Fig. 3 Coronal, sagittal and transaxial images of unconscious pig obtained using SPECT scanner at 2 hr, 3 hr and 4 hr after intragastric injection of ^{99m}Tc-pertechnetate, where color intensity is proportional to pixel activity (percentage)

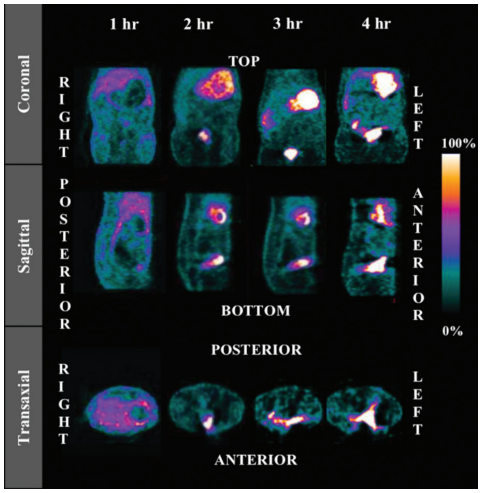

Fig. 4 Coronal, sagittal and transaxial images of conscious pig obtained using SPECT scanner at 1 hr, 2 hr, 3 hr and 4 hr after intravenous injection of ^{99m}Tc-pertechnetate, where color intensity is proportional to pixel activity (percentage)

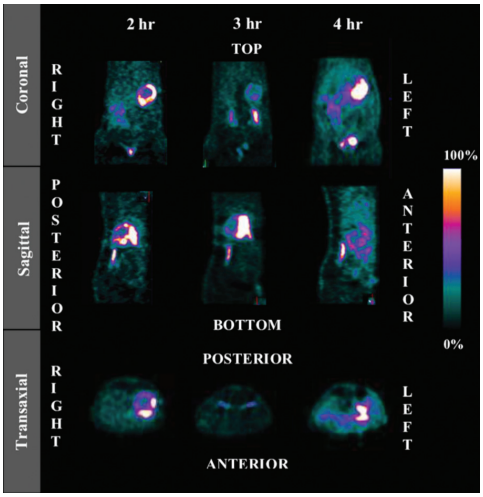

Fig. 5 Coronal, sagittal and transaxial images of conscious pig obtained using SPECT scanner at 2 hr, 3 hr and 4 hr after intragastric injection of ^{99m}Tc-pertechnetate, where color intensity is proportional to pixel activity (percentage)

Comparative qualitative distribution of ^{99m}Tc following IG injection in the unconscious pig was observed. Fig. 3 shows the SPECT image viewing at 2 hr, 3 hr and 4 hr. The ^{99m}Tc-pertechnetate uptake in the stomach was clearly seen in the fundus and antrum area soon after 2 hr of injection. However, the images showed that the activity of distribution remained in the stomach after 3 hr and 4 hr, while no activity was found in the duodenum. The absence of radioactivity in the duodenum might have been due to the effects of isoflurane on gastrointestinal motility.

This result was consistent with a previous study which reported that the gastrointestinal motility in pigs decreased with increasing isoflurane concentration (Anderson et al., 2002). Another study reported that the gastrointestinal transit of charcoal in rats was reduced by approximately 50% at 120 min after brief isoflurane anesthesia (Torjman et al., 2005). This could be explained by isoflurane inducing the disruption of normal gastrointestinal motility by inhibition of intestinal smooth muscle contractions (Dryn et al., 2018).

According to the current protocol to observe whether unconscious and conscious conditions in pigs affect the bio-distribution of ^{99m}Tc−pertechnetate, comparative SPECT viewing in conscious pigs after IV and IG injections are presented in Figs. 4 and 5, respectively.

As illustrated in Fig. 4, the distribution of ^{99m}Tc activity after the first hour of IV administration in conscious pigs indicated that it was rapidly taken up by the gastric mucosa and marginally displayed in the peritoneal cavity. After 2 hr, the radiotracer was distributed and accumulated in the gastric mucosa, whereas some activities were found in the bladder. In the following third and fourth hours, ^{99m}Tc-pertechnetate activity involved re-entry and increased activity in the stomach, while radiotracer uptake occurred more gradually in the bladder. These results were consistent with earlier results where isofurane-induced unconscious pigs were administrated via the IV route and could be explained by the effect of isoflurane on the gastrointestinal motility, which would probably not affect the biodistribution of ^{99m}Tc-pertechnetate in pigs following IV administration.

Following IG injection in conscious pigs, the behavior of the radiotracer is shown in Fig. 5. After 2 hr, there was higher uptake of ^{99m}Tc in the stomach than in the duodenum and bladder. After 3 hr, the main activity was stored in the stomach and some activity was transferred to the small intestine, kidneys and bladder, respectively. After 4 hr, the ^{99m}Tc activity increased in the order of the small intestine > kidneys > bladder. This observation was inconsistent with earlier investigation when the pig was induced to an unconscious state using isoflurane, confirming that isoflurane influenced the gastrointestinal motility in pigs. Evidence of activity in the kidneys and bladder in a conscious pig suggests the clearance profile of ^{99m}Tc via the kidneys and bladder.

The use of ^{99m}Tc-pertechnetate is one clinical radiopharmaceutical in the imaging of different organs. The current study confirmed the regular distribution of ^{99m}Tc-pertechnetate when intravenously administered under both unconscious and conscious conditions. Visualization of ^{99m}Tc-pertechnetate via intragastric administration in animals with isoflurane-induced unconsciousness status tended to present an irregular bio-distribution pattern. This behavior might have been due to the effect of isoflurane on the reduction of gastrointestinal motility in pigs. This study may possibly further improve the efficient use of available ^{99m}Tc imaging for both IV and IG administration experiments in pigs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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