Tohoku J. Exp. Med., 2007, **211**, 379-385

On-Site Preparation of Technetium-99m Labeled Human Serum Albumin for Clinical Application

Yuh-Feng Wang,[1,2] Mei-Hua Chuang,[1,3] Jainn-Shiun Chiu,[2] Thau-Ming Cham[1] and Mei-Ing Chung[1]

[1]Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan
[2]Department of Nuclear Medicine, Buddhist Dalin Tzu Chi General Hospital, Chia-Yi, and College of Medicine, Tzu Chi University, Hualien, Taiwan
[3]Department of Pharmacy, Buddhist Dalin Tzu Chi General Hospital, Chia-Yi, Taiwan

Wang, Y.F., Chuang, M.H., Chiu, J.S., Cham, T.M. and Chung, M.I. *On-Site Preparation of Technetium-99m Labeled Human Serum Albumin for Clinical Application*. Tohoku J. Exp. Med., 2007, **211** (4), 379-385 —— Technetium-99m labeled human serum albumin (Tc-99m HSA) is an important radiopharmaceutical for clinical applications, such as cardiac function tests or protein-losing gastroenteropathy assessment. However, because of transfusion-induced infectious diseases, the safety of serum products is a serious concern. In this context, serum products acquired from patients themselves are the most ideal tracer. However, the development of rapid separation and easy clinical labeling methods is not yet well established. Under such situation, products from the same ethnic group or country are now recommended by the World Health Organization as an alternative preparation. This article describes the on-site preparation of Tc-99m HSA from locally supplied serum products. Different formulations were prepared and the labeling efficiency and stability were examined. Radio-labeling efficiencies were more than 90% in all preparation protocols, except for one that omitted the stannous solution. The most cost-effective protocol contained HSA 0.1 mg, treated with stannous fluoride 0.2 mg, and mixed with Tc-99m pertechnetate 30 mCi. A biodistribution study was performed in rats using a gamma camera immediately after intravenous administration of radiolabeled HSA. Tissue/organ uptake was obtained by measuring the radioactivity in organs after sacrificing the rats at timed intervals. The biologic half-life was about 32 min, determined from sequential venous blood collections. These data indicate that our preparation of Tc-99m HSA is useful and potentially applicable clinically. In addition, this on-site preparation provides the possibility of labeling a patient's own serum for subsequent clinical application. ——
Technetium-99m; albumin; radiopharmaceutical; infection; biodistribution
© 2007 Tohoku University Medical Press

Albumin is a water-soluble polypeptide, synthesized in the liver, of about 576 amino acids, with a molecular mass of about 68,000 daltons (Atkins et al. 1980). Human serum albumin (HSA) accounts for more than 50% of the plasma protein and is also the major component of plasma

Received November 13, 2006; revision accepted for publication March 2, 2007.
Correspondence: Mei-Ing Chung, Ph.D., Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, 100 Shi-Chuan 1st Rd., Kaohsiung, Taiwan.
e-mail: meinch@kmu.edu.tw

protein at a concentration of about 35-50 gm/l in healthy adults (Quinlan et al. 2005; Hankins 2006). The primary function of the albumin is to provide colloid osmotic pressure at the capillary membrane, which in turn prevents plasma loss from the capillaries and thus maintains the plasma volume. In addition to the osmotic properties, HSA also functions as a carrier for hormones, enzymes, fatty acids, metal ions, or medicinal products (Anker and Coats 1999; Haak 1999; Johnson 1999; Schussler 2000), and as a free-radical scavenger (Evans 2002). A decreased serum level of HSA may be caused by inability to synthesize albumin, which is a predominant feature of chronic liver disease; increased catabolism, such as inflammatory processes; or unknown loss from the urinary or gastrointestinal tract.

HSA labeled with technetium-99m ($99mTcO_4^-$) given intravenously, is distributed throughout the body in much the same way as the patient's own serum albumin, and serves as a suitable tracer with which to transiently image the vascular compartment. Tc-99m HSA is an important radiopharmaceutical applied clinically (Thrall et al. 1978; Atkins et al. 1980; Kelly et al. 1992), for cardiac function tests (Ferraro et al. 1996; Vanhove et al. 2002) or assessment of protein-losing gastroenteropathies (Miyata et al. 2000; Chiu et al. 2001; Hung et al. 2002). Commercially-available HSA comes as a powder or crystal extracted from blood. When mixed with Tc-99m pertechnetate, it forms Tc-99m HSA. However, because of transfusion-induced infectious diseases, such as human immunodeficiency virus (HIV) infection, viral hepatitis, and Creutzfeldt-Jakob disease, the safety of serum products is a serious concern. Therefore, the use of the native people's own serum products has been recommended by the World Health Organization (WHO).

In fact, a cold kit of HSA for the preparation of Tc-99m HSA has not been available for use in clinical practice for some time. Therefore, we undertook this project to formulate a procedure for the on-site preparation of Tc-99m HSA. The purpose of this study was to compare the stability and efficiency of the preparation of Tc-99m-labeled albumin from a serum product of domestic origin. After determining an efficient and cost-effective protocol, we performed animal studies of biodistribution and tests for sterility and pyrogenicity.

Material and Methods

Preparation of the Tc-99m labeled HSA

HSA ("CBSF" [20%] Human Albumin Solution) was obtained from the Chinese Blood Services Foundation. This product contains 20% protein, of which at least 95% is albumin. The components of this product come from domestic donors after appropriate screening for infectious diseases, such as HIV infection or hepatitis. The HSA was divided into aliquots of 2 ml/vial (0.4 mg/vial) and stored in a 20°C freezer.

One day (at least 8 hrs) before labeling, frozen HSA was moved from the freezer to a 4°C refrigerator. Sodium pertechnetate (Tc-99m pertechnetate) was eluted from a Mo-99/Tc-99m generator (Ultra-Techne Kow®; Daiichi Radioisotope Laboratories, LTD., Tokyo), following instructions provided by the manufacturer. Stannous solution (Amerscan™ Stannous Agent, Amersham plc, Buckinghamshire, UK) was freshly prepared, before labeling, by carefully adding 10 ml of normal saline to the kit vial. The final concentration of the stannous fluoride in the stannous solution is 0.4 mg/ml.

Labeling was done by mixing HSA with the stannous solution and Tc-99m pertechnetate to provide five different concentrations of HSA and stannous solution. First, the desired amount of HSA was measured out and placed in a sterile tube (BD Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Next, the desired amount of stannous solution was transferred to the appropriate tube using volumetric pipettes. Mixing was performed manually by gently shaking the tubes to avoid bubble formation. Tc-99m pertechnetate was eluted and counted by a dose calibrator (CRC®-15R; Capintec, INC., NJ, USA) to the desired dosage and placed in sterile tubes. The HSA-stannous mixtures were then transferred aseptically to the tubes containing 30 mCi of Tc-99m pertechnetate using a syringe and carefully delivering the solution down the side wall of the tubes. Mixing was performed manually by shaking the tubes gently to avoid bubble formation.

Five preparation protocols were examined. The compositions were: (A) HSA 0.1 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi; (B) HSA 0.1 mg, stannous solution 0 ml, and Tc-99m pertechnetate 30

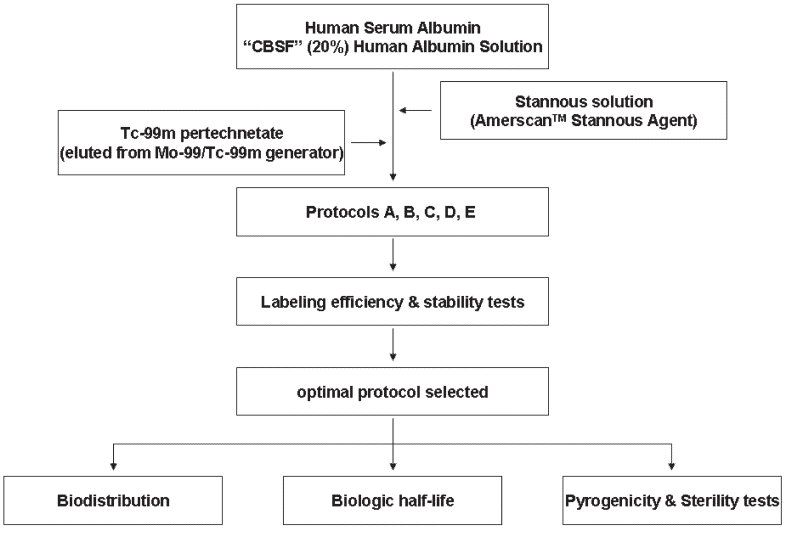

Fig. 1. The scheme for on-site preparation of Tc-99m HSA for clinical application.

mCi; (C) HSA 0.1 mg, stannous solution 1.0 ml, and Tc-99m pertechnetate 30 mCi; (D) HSA 0.2 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi; and (E) HSA 0.4 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi (Fig. 1).

Labeling efficiency and stability

Labeling efficiency and stability were determined by means of thin layer chromatography (TLC) (Fu et al. 2004) analysis using a commercial quality control kit (Tec-Control, Mayjoy Co., CA, USA) and developed by acetone.

Biodistribution

A gamma camera (DST-XLi, General Electric Medical Systems, Buc, France) was used to view rats (Wistar strain, male, weighing from 200 to 250 gm) that had been injected, via the tail vein, with 2 mCi of Tc-99m HSA. The rats were anesthetized with an intraperitoneal injection of pentobarbital (0.006 mg/100 gm). The amount of HSA injected was 0.05 mg. The gamma camera was fitted with a low energy/high resolution collimator, and the images were processed by a gamma-camera working station (POWERstation SPX, IBM RS6000, Vision 5.2.0, General Electric Medical Systems). Each rat was studied by taking 750 frames of 0.4 sec, followed by 1 frame of 60 sec (total study time, about 6 min).

In addition to the imaging studies, an examination of biodistribution changing over time was performed. Rats were maintained in a fixed position using tubes (cylinders) large enough for them to slide into; radio-labeled HSA was injected via a tail vein. The animals were sacrificed by direct decapitation at 10, 30, 60, 120, and 240 min after administration of radio-labeled HSA, followed by dissection. Organs and tissues were separated, and the radioactivity (cpm/gm of organ) was determined using a gamma counter (COBRA^{TM}II, Packard, Netherlands).

Blood clearance of radio-labeled HSA

Blood clearance studies were performed to understand the in vivo behavior of the injected Tc-99m HSA. Rats (n = 5) without previous administration of any radiopharmaceutical were acquired. The radio-labeled preparations were diluted with normal saline to a concentration of 74 MBq/ml (2 mCi/ml), and 0.2ml of this preparation was injected into the proximal part of the tail vein. At fixed time intervals (5, 10, 15, 30, 60, and 120 min); 0.2 ml samples of blood were withdrawn from the distal end of the tail vein. All of the samples were counted in a gamma well counter and were compared with a same value standard radiopharmaceutical. The blood clearance rates were calculated by means of the logarithmic equation as "Percentage = $q \times$ [log(Time)] + b", where "Percentage" is the percentage of residual radioactivity; "Time" is the minutes after initial of study; "q" and "b" are slope and intercept, respectively.

Pyrogenicity and Sterility Testing

Pyrogenicity was determined using a bacterial endotoxin test called the *Limulus Amebocyte Lysate (LAL) test*

(Vanhaecke et al. 1987). The sensitivity of the LAL reagent is 0.25 endotoxin units (EU)/ml. According to the requirement, as specified in *USP27/NF22* (U.S. Pharmacopeia 2004), the limit with regard to endotoxin content of the on-site Tc-99m HSA preparation is 175/V USP EU/ml of the injection, where V is the maximum recommended dose in milliliters (U.S. Pharmacopeia 2004a).

The sterility of our on-site Tc-99m HSA preparation was tested, as stated in *USP27/NF22* (U.S. Pharmacopeia 2004b). One ml of our preparation was added to a test tube containing 15 ml of SCD and TGC medium. The tubes were observed for turbidity and results were recorded every day for 14 days. A negative result was indicated by a clear culture medium (no turbidity noted) over the 14 days of observation.

Ethics

All experiments were performed in accordance with the Animal Protection Act of the Council of Agriculture, and were approved by the Institutional Animal Care and Use Committee of Dalin Tzu Chi General Hospital.

Results

Stability of radiolabeling

Radiolabeling efficiencies were greater than 90% for all the preparation protocols, except for Protocol B, which contained no stannous solution (Fig. 2). Up to 24 hrs after the initial preparation, the percentage of HSA labeled remained over 70%. Among the protocols tested, based on cost/performance, we chose 0.1 mg HSA plus 0.5 ml stannous solution and 30 mCi Tc-99m pertechnetate (Protocol A) for its biodistribution, biologic half-life, and results of pyrogenicity and sterility studies.

Biodistribution

When Tc-99m HSA is injected intravenously into a rat, the main vessels, the heart, the liver, and the kidneys initially were visualized (Fig. 3). In addition, rats were sacrificed in time sequence (10, 30, 60, 120, and 240 min). Radio-counts of

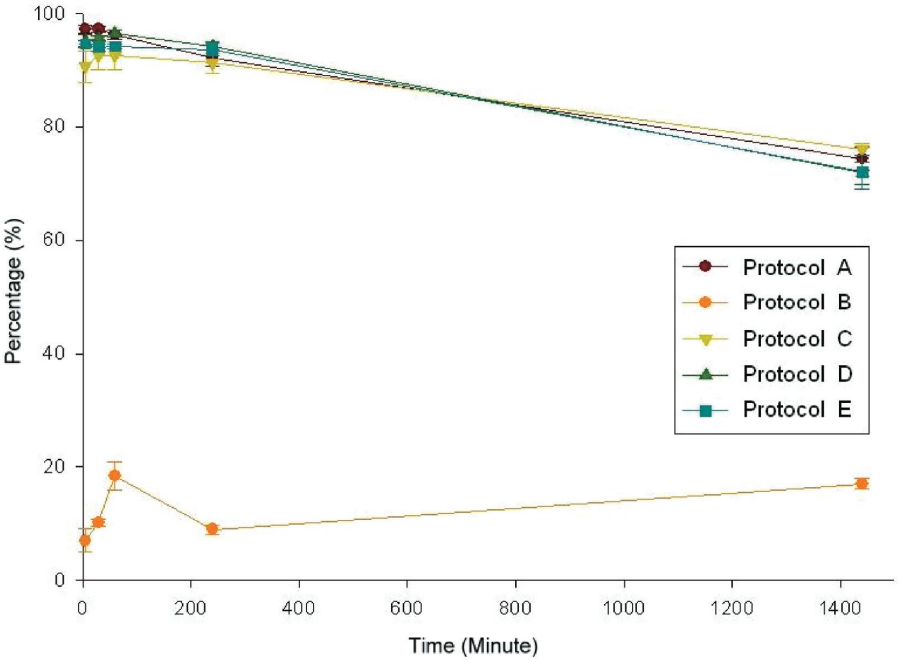

Fig. 2. Labeling efficiency and stability test of all the preparation protocols.
Protocol A: HSA 0.1 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi;
Protocol B: HSA 0.1 mg, stannous solution 0 ml, and Tc-99m pertechnetate 30 mCi;
Protocol C: HSA 0.1 mg, stannous solution 1.0 ml, and Tc-99m pertechnetate 30 mCi;
Protocol D: HSA 0.2 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi;
Protocol E: HSA 0.4 mg, stannous solution 0.5 ml, and Tc-99m pertechnetate 30 mCi.

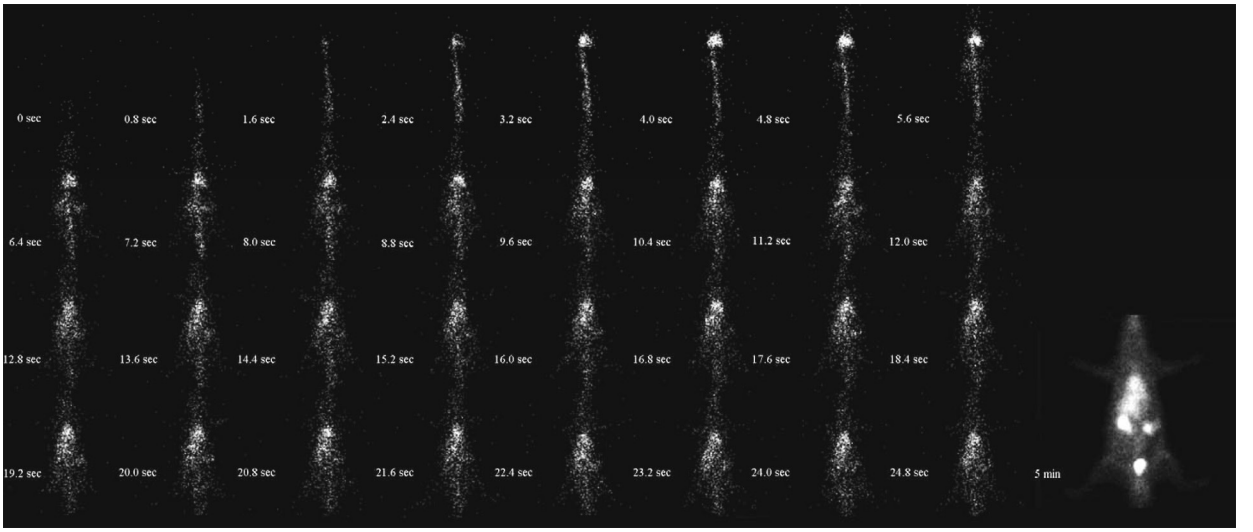

Fig. 3. Biodistribution study of the on-site prepared Tc-99m HSA. The scintigraphy was obtained after intravenous injection of the radiolabeled HSA in the tail vein of a rat. From upper left: dynamic images at 0 to 24.8 sec after initiation of the study. Lower right: enlarged image taken at 5 min after injection.

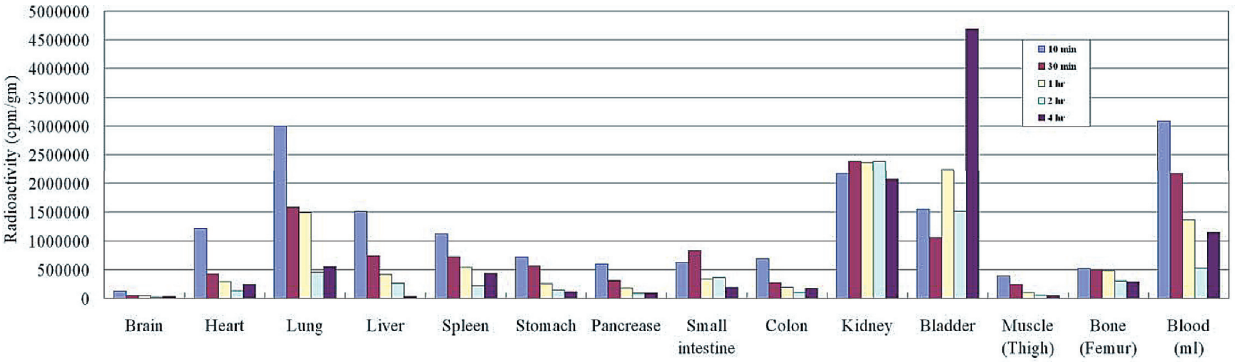

Fig. 4. Time course of tissue distribution of Tc-99m HSA in normal rats.

organ (tissue)/gm were calculated for brain, heart, lung, liver, spleen, stomach, pancreas, small intestine, colon, kidneys, urinary bladder, muscle (thigh), bone (femur), and blood (Fig. 4). These investigations demonstrated that most of the injected dose was distributed within blood, followed by the lung, liver, and kidneys. Little uptake was evident in the brain.

Blood clearance

The blood clearance rates of this radiolabeled HSA are shown in Fig. 5. A decline in blood clearance was observed over time. The subsequent formula is "Percentage = 0.4571 – 0.2712 × log (Time)" and the biological half-live observed was about 32 min (31.998 min).

Pyrogenicity and Sterility Testing

At the end of the 6-hr evaluation in the blood clearance study, left-over radiolabeled HSA was collected for the pyrogenicity and sterility tests (n = 5). In the pyrogenicity tests, after diluting 50-fold and 83-fold, the specimen yielded negative results. In addition, after being incubated for 14 days, our culture medium still presented a clear appearance. All the samples were free from pyrogenicity and all passed the sterility tests.

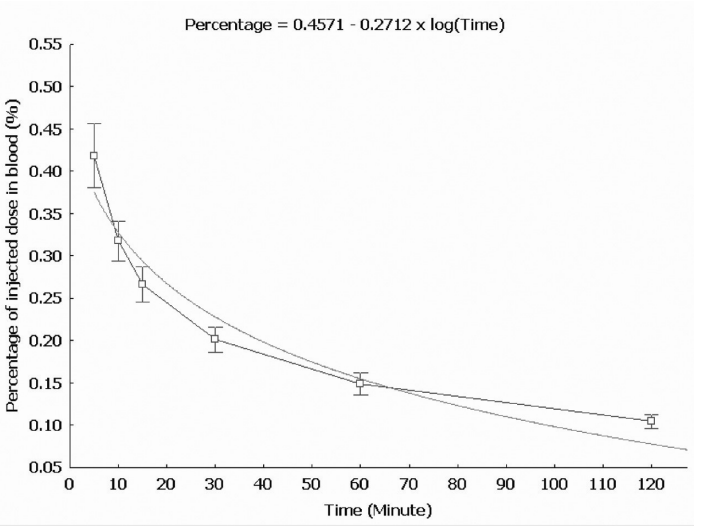

Fig. 5. Blood retention of the radiolabeled HSA.

Discussion

The formerly available HSA cold kits contain a lyophilized mixture of human albumin and stannous agent ($SnC_4H_4O_6$). In our preparation protocol, 0.1 mg of HSA solution and 0.5 ml of stannous solution provide adequate labeling efficiency and stability. Since the stannous solution commonly used is readily available for commercial application, we used Amerscan™ Stannous Agent to reduce Tc-99m pertechnetate in the preparation of radiolabeled HSA (Fu et al. 2005). Our results demonstrate that a labeling efficiency satisfactory for clinical application can be achieved.

An ideal tracer for radionuclide blood-pool scintigraphy should be prepared easily and retained in the blood. HSA is one of the ideal constituents. Tc-99m HSA has been in use in nuclear medicine for almost 30 years. In comparison with Tc-99m labeled RBC, Tc-99m labeling of albumin is much easier and safer, and has a stable and high-labeling yield that is independent of the patient's medication. Nevertheless, the clearance of Tc-99m HSA from the plasma is rapid, which compromises its clinical usefulness. In fact, biological behavior may be variable in different labeling kits (Kristensen 1986; Vanbilloen et al. 1993). For our on-site preparation, the blood clearance rates of this radiolabeled HSA are shown in Fig. 5, and the biological half-life observed was about 32 min.

Because of the risk of transmission of infectious diseases through blood components, it is best to select voluntary, non-remunerated donors from low-risk populations, who give blood on a regular basis, as these individuals are at a lower risk of transmitting transfusion-transmissible infections than are family/replacement donors, or paid donors. However, even with the most careful selection, some donors may be seropositive for HIV or other infectious agents. Given this circumstance, the WHO has recommended the use of the products for domestic supplied since 1975. In accordance with this concern, we prepared Tc-99m HSA in the laboratory, using a serum product of Taiwanese origin. Our results showed that on-site preparation of Tc-99m-labeled HSA for domestic serum products is achievable, and we believe that this result can be applied to other nuclear medicine laboratories in other countries.

Conclusion

Tc-99m HSA has been in use in nuclear medicine for almost 30 years. The main problem with the clinical application of this radiopharmaceutical is the risk of unknown infections. In this study, we prepared Tc-99m HSA in the laboratory using a serum product of native origin. Our data indicate that on-site preparation of Tc-99m HSA

is easy, achieving highly specific activity of labeled protein, as well as extended in vitro stability. This product may be clinically applicable.

Acknowledgments

This research was supported by a research grant of the Buddhist Dalin Tzu Chi General Hospital (DTCRD 92[2]-17).

References

Anker, S.D. & Coats, A.J. (1999) Cardiac cachexia: a syndrome with impaired survival and immune and neuroendocrine activation. *Chest*, **115**, 836-847.

Atkins, H.L., Klopper, J.F., Ansari, A.N., Meinken, G., Richards, P. & Srivastava, S.C. (1980) A comparison of Tc-99m-labeled human serum albumin and in vitro labeled red blood cells for blood pool studies. *Clin. Nucl. Med.*, **5**, 166-169.

Chiu, N.T., Lee, B.F., Hwang, S.J., Chang, J.M., Liu, G.C. & Yu, H.S. (2001) Protein-losing enteropathy: diagnosis with (99m)Tc-labeled human serum albumin scintigraphy. *Radiology*, **219**, 86-90.

Evans, T.W. (2002) Albumin as a drug: biological effects of albumin unrelated to oncotic pressure. *Aliment. Pharmacol. Ther.*, **16**, Suppl. 5, 6-11.

Ferraro, S., Perrone-Filardi, P., Desiderio, A., Betocchi, S., D'Alto, M., Liguori, L., Trimigliozzi, P., Turco, S. & Chiariello, M. (1996) Left ventricular systolic and diastolic function in severe obesity: a radionuclide study. *Cardiology*, **87**, 347-353.

Fu, C.M., Wang, Y.F., Chao, Y.C., Hung, S.H. & Yang, M.D. (2004) Directly Labeling Ferrite Nanoparticles With Tc-99m Radioisotope for Diagnostic Applications. *IEEE. T. Magn.*, **40**, 3003-3005.

Fu, C.M., Wang, Y.F., Guo, Y.F., Lin, T.Y. & Chiu, J.S. (2005) *In vivo* Bio-Distribution of Intravenously Injected Tc-99m Labeled Ferrite Nanoparticles Bounded with Biocompatible Medicals. *IEEE T. Magn.*, **41**, 4120-4122.

Haak, T. (1999) New developments in the treatment of type 1 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes*, **107**, S108-113.

Hankins, J. (2006) The role of albumin in fluid and electrolyte balance. *J. Infus. Nurs.,* **29**, 260-265.

Hung, J.C., Gadient, K.R., Mahoney, D.W. & Murray, J.A. (2002) In-house preparation of technetium 99m-labeled human serum albumin for evaluation of protein-losing gastroenteropathy. *J. Am. Pharm. Assoc.*, **42**, 57-62.

Johnson, A.M. (1999) Low levels of plasma proteins: malnutrition or inflammation? *Clin. Chem. Lab. Med.*, **37**, 91-96.

Kelly, M.J., Cowie, A.R., Antonino, A., Barton, H. & Kalff, V. (1992) An assessment of factors which influence the effectiveness of the modified in vivo technetium-99m-erythrocyte labeling technique in clinical use. *J. Nucl. Med.*, **33**, 2222-2225.

Kristensen, K. (1986) Biodistribution in rats of 99mTc-labelled human serum albumin. *Nucl. Med. Commun.*, **7**, 617-624.

Miyata, M., Yoshida, M., Saka, M. & Kasukawa, R. (2000) Protein-losing gastroenteropathy in system lupus erythematosus: diagnosis with 99mTc-human serum albumin scintigraphy. *Arthritis Rheum.*, **43**, 1900.

Quinlan, G.J., Martin, G.S. & Evans, T.W. (2005) Albumin: biochemical properties and therapeutic potential. *Hepatology*, **41**, 1211-1219.

Schussler, G.C. (2000) The thyroxine-binding proteins. *Thyroid*, **10**, 141-149.

Thrall, J.H., Freitas, J.E., Swanson, D., Rogers, W.L., Clare, J.M., Brown, M.L. & Pitt, B. (1978) Clinical comparison of cardiac blood pool visualization with technetium-99m red blood cells labeled in vivo and with technetium-99m human serum albumin. *J. Nucl. Med.*, **19**, 796-803.

U.S. Pharmacopeia (2004a) Bacterial endotoxin test. In: *USP27/NF22*, United States Pharmacopeia and National Formulary, The United States Pharmacopeial Convention Inc. 2004, pp. 2169-2173.

U.S. Pharmacopeia (2004b) Sterility test. In: *USP27/NF22*, United States Pharmacopeia and National Formulary, The United States Pharmacopeial Convention Inc. 2004, pp. 2157-2162.

Vanbilloen, H.P., Verbeke, K.A., De Roo, M.J. & Verbruggen, A.M. (1993) Technetium-99m labelled human serum albumin for ventriculography: a comparative evaluation of six labelling kits. *Eur. J. Nucl. Med.*, **20**, 465-472.

Vanhaecke, E., Pijck, J. & Vuye, A. (1987) Endotoxin testing. *J. Clin. Pharm. Ther.*, **12**, 223-225.

Vanhove, C., Walgraeve, N., De Geeter, F. & Franken, P.R. (2002) Gated myocardial perfusion tomography versus gated blood pool tomography for the calculation of left ventricular volumes and ejection fraction. *Eur. J. Nucl. Med. Mol. Imaging.*, **29**, 735-741.