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In vitro evaluation of canine leukocytes radiolabeled in whole blood with ^{99m}Tc stannous colloid

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Abstract

Introduction: Technetium-99m stannous colloid (^{99m}TcSnC)-labeled leukocytes are used to investigate a variety of inflammatory diseases in human medicine. The present study investigates the in vitro behavior of canine leukocytes labeled in whole blood with ^{99m}TcSnC.
Methods: Blood samples from 10 healthy dogs were labeled with ^{99m}TcSnC using a standard procedure. The distribution of radioactivity among blood components (plasma, leukocyte layers and erythrocytes) was measured following separation of the radiolabeled samples across Histopaque density gradients. Phagocytic function of labeled and unlabeled leukocytes was estimated using zymosan particles. Labeling retention by leukocytes was determined at 1, 3, 4 and 7 h postlabeling.
Results: The mean±standard error percentage of radioactivity associated with plasma, erythrocyte and leukocyte fractions was 2.0±0.21%, 55.5±0.60% and 42.5±0.54%, respectively (the last comprising 70.2±0.83% in polymorphonuclear leukocytes and 29.8±0.83% in mononuclear leukocytes). Labeled canine leukocytes had a phagocytic activity of 91.3±0.28% (control, 91.7±0.26%). The radiolabeled canine leukocytes retained 94.1±0.30% of radioactivity at 7 h postlabeling.
Conclusions: Radiolabeling of canine leukocytes in whole blood with ^{99m}TcSnC has minor adverse effect on their phagocytic function. The radiolabeled canine leukocytes retained a large percentage of radioactivity for at least 7 h postlabeling.

Keywords: ^{99m}TcSnC; Radiolabeling canine leukocytes; Labeling efficiency; Phagocytic function; Labeling retention

1. Introduction

Radiolabeling of autologous leukocytes to localize inflammatory foci is a well-established diagnostic technique in human medicine. The technique is based on the principle that leukocytes accumulate at sites of inflammation. Scintigraphic imaging of a patient whose leukocytes have been radiolabeled allows the visualization of inflammatory foci. Leukocytes can be radiolabeled indirectly (in vivo) or directly (in vitro) [1,2]. Indirect radiolabeling can be achieved by targeting leukocyte antigens or receptors by intravenous injection of radiolabeled monoclonal antibodies [3,4], chemotactic peptides [5], cytokines such as interleukin-1 [6] and interleukin-2 [7] and platelet factor-4 [8]. Direct radiolabeling of leukocytes involves collection of blood from the patient, radiolabeling in vitro using such radiopharmaceuticals as Technetium-99m stannous colloid (^{99m}TcSnC), ^{99m}Tc-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) and indium-111 oxine (^{111}In-oxine) [1,2] and reinjection of the radiolabeled leukocytes into the patient.

The use of ^{99m}TcSnC to radiolabel human leukocytes was first reported in the early 1980s [9]. Although ^{99m}TcSnC has not been approved as an inflammatory imaging agent in human nuclear medicine in the United States [10], it has been successfully used in Australia over the last 20 years to investigate a variety of inflammatory processes in humans [11–17].

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The exact mechanism by which human leukocytes are labeled by ^{99m}TcSnC is not clear. A number of authors have reported that all leukocytes become labeled with ^{99m}TcSnC by engulfment of colloid particles, that is, by true phagocytosis [11,12,14]. Other authors have stated that only neutrophils phagocytose ^{99m}TcSnC, whereas other blood cells such as monocytes and erythrocytes reversibly bind ^{99m}TcSnC at their surfaces with lower affinity [18]. Another study found that ^{99m}TcSnC adheres to the surface of all the leukocytes and phagocytosis is not involved [19]. A recent study has demonstrated that the uptake of ^{99m}TcSnC by neutrophils takes place via phagocytosis directly related to the complement receptor 3 [20].

The reported labeling efficiency of ^{99m}TcSnC in human whole blood is greater than 95% [14,16,21–23]. However, the labeling efficiency of ^{99m}TcSnC is affected by many factors and optimal labeling occurs with colloid particles of 1–3 μm diameter [12]. The purity of the starting materials, the speed of the rotator that mixes the radiolabeled sample, the concentration of heparin in the blood sample, the freshness of the colloid preparation and the incubation time are other factors that affect the labeling efficiency [10,12]. Previous in vitro studies have demonstrated that radiolabeling human leukocytes with ^{99m}TcSnC in either whole blood or leukocyte-rich plasma (LRP) does not affect leukocyte viability or phagocytic function [11,18,20].

The main advantage of ^{99m}TcSnC over other in vitro labeling radiopharmaceuticals is its ability to radiolabel leukocytes in whole blood without the need to separate the leukocytes. This makes radiolabeling leukocytes with ^{99m}TcSnC a simple, fast and safe procedure [11–17]. These advantages together with the lower cost of ^{99m}TcSnC compared with other radiopharmaceuticals are of particular benefit in veterinary nuclear medicine.

Radiolabeling canine leukocytes with ^{99m}TcSnC has been reported in whole blood [24] and in LRP [25], and high levels of radiolabeling efficiency and leukocyte viability were demonstrated in both studies. ^{99m}TcSnC-radiolabeled leukocytes have been safely used in healthy dogs to radiolabel leukocytes and have successfully localized induced abscesses in limited numbers of animals [25]. However, the effect of the radiolabeling procedure using ^{99m}TcSnC on the phagocytic function of canine leukocytes has not been investigated.

The objectives of the present study were to investigate the distribution of radioactivity among blood components, to study the phagocytic function of radiolabeled canine leukocytes and to determine the labeling retention of the leukocytes.

2. Methods

2.1. Animals

Ten clinically healthy adult greyhounds of mixed age and gender were used in the study. The dogs were retiring blood donors housed long term in the Animal House at the Faculty of Veterinary Science, The University of Melbourne. The dogs were examined daily by a registered veterinarian. Approval for the use of the dogs was received from the Animal Ethics Committee of The University of Melbourne.

2.2. Preparation of ^{99m}TcSnC

The radiopharmaceutical was prepared using a commercially available stannous colloid cold kit (Radpharm Scientific, Canberra, Australia). The kit consisted of two vials (A and B). Vial A contained 5 ml of pyrogen-free sodium fluoride (1 mg/ml) in sterile distilled water. Vial B contained 640 μg of stannous colloid as a lyophilized powder. Vials A and B were combined at room temperature and mixed thoroughly until all the lyophilized powder was dissolved. A 3-ml volume of the stannous colloid mixture was drawn into a 5-ml syringe, and 0.25 ml was then filtered through a 0.20-μm microfilter (Sartorius, Goettingen, Germany). ^{99m}TcSnC was prepared by adding 1 GBq of ^{99m}Tc sodium pertechnetate (Ansto Radiopharmaceuticals and Industries, Menai, Australia) to the 0.25 ml of filtered stannous colloid. Sterile normal saline (0.9% NaCl) (Baxter, Old Toongabbie, Australia) was added to give a total volume of 1.5 ml. The preparation was then incubated at room temperature for 60 min at 2×*g* on a rotating mixer (Ratek Instruments, Boronia, Australia).

2.3. Measurement of the distribution of radioactivity among blood components and between leukocyte subpopulations

A 5-ml blood sample was collected from a jugular vein of each dog into a syringe containing 50 IU sodium heparin (Pfizer, Auckland, New Zealand). A total of 1 ml (0.5 GBq) ^{99m}TcSnC was added to each blood sample. The sample was incubated at room temperature for 60 min at 2×*g* on a rotating mixer.

A 2-ml volume of each radiolabeled blood sample was placed in a 10-ml plastic tube containing a Histopaque double density gradient solution, consisting of Histopaque-1077 (Sigma Aldrich, Castle Hill, Australia) overlying Histopaque-1119 and centrifuged at 600×*g* for 30 min at room temperature. Centrifugation was terminated without applying brakes, to avoid disturbing the leukocyte layers.

After centrifugation, the total radioactivity of the sample was measured using a dose calibrator (Atomlab 100; Gammasonics, Canberra, Australia). The plasma was then collected from each sample, and its radioactivity was measured. The mononuclear cell layer (at the interface between the plasma and the Histopaque-1077 layer) was aspirated and resuspended in 3 ml of phosphate-buffered saline (PBS). The polymorphonuclear cell layer (at the interface between the Histopaque-1077 and Histopaque-1119 layers) was then collected and resuspended in 3 ml of PBS. To further purify the polymorphonuclear layer, we added 6 ml of cold distilled water (4°C) to the cell preparation to lyse any residual erythrocytes. After 30 s of