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RESEARCH ARTICLE

Preparation of ^{131}I-Pyrimethamine and evaluation for scintigraphy of experimentally *Toxoplasma gondii*-infected rats

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Abstract

We aimed to assess the ability of ^{131}I-Pyrimethamine scintigraphy to detect the lesions of *Toxoplasma gondii* infection. An experimental model of toxoplasmosis was developed. The presence of toxoplasmosis was confirmed 60 days after implantation. Pyrimethamine was radioiodinated with I-131. The radioligand was validated by the requisite quality control tests to check its radiolabeling efficiency, in vitro stability and radiochemical purity etc. ^{131}I-Pyrimethamine (specific activity: 7.08 MBq/μmol) was injected intravenously into the tail vein of the control and infected rats. Static whole body images of the rats were acquired under the gamma camera at 5 min, 45 min, 2 h, 6 h, and 24 h following the intravenous administration of the radioactivity (3.7 MBq/rat). Then the scintigraphic data were analyzed both visually and semiquantitatively. Regions of interest (ROIs) were drawn over the organs (thyroid, stomach, liver, bladder, and soft tissues) to calculate the ratios of the radiotracer in infected vs. control rats. The mean ratio of radiotracer in infected/control rats in the liver and diaphragm was over 1 at 45 min which persisted till 24 h. In conclusion, ^{131}I-Pyrimethamine may be useful agent for diagnosis toxoplasmosis especially involving liver and diaphragm, needs further preclinical validation before being extended for use in clinical applications.

Keywords: *Toxoplasma gondii*, toxoplasmosis, Wistar rat, 131I-Pyrimethamine, radionuclide imaging

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Introduction

Toxoplasma gondii (*T. gondii*), the obligate intracellular parasite is the causative agent of toxoplasmosis. Unlike the other apicomplexan parasites, *T. gondii* has a broad host range and is able to replicate within all nucleated vertebrate cell types investigated thus far. Replication occurs within the parasitophorous vacuole, which expands in size during the course of infection until the host cell finally ruptures and releases the parasites which can then actively invade new host cells (Barragan & Sibley, 2003). Recent studies have demonstrated that wild rodents can represent not only a highly prevalent, but also a persistent intermediate host reservoir for *T. gondii* (Jackson et al., 1986; Webster 1994). For example, a breeding rat population derived from wild caught individuals, maintained in an enclosure which prevented any contact with cats and hence direct oocysts contamination, was found to maintain a *T. gondii* infection prevalence similar (at a mean of 35%) to that of wild populations (Webster 1994). Infected rats

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have also been suggested as animal models for human toxoplasmosis because toxoplasmosis in both species is often subclinical. Much of the literature on toxoplasmosis in rats is 30–50 years old and usually unknown to contemporary researchers (Dubey & Frenkel, 1998).

The diagnosis of toxoplasmosis mainly depends on serologic methods. Although there are many advances in molecular diagnosis, sometimes the results may be confusing (Villard et al., 2012). Focusing on involved organ may be difficult by such methods.

The Pyrimethamine is one of the most effective antiprotozoal of the imidazole group and widely used in medicine and veterinary science for the treatment of toxoplasmosis (Barbosa et al., 2012; Romand et al., 1993; Derouin et al., 1992).

The aim of this study was to assess the ability to detect the lesions of toxoplasmosis infection by using ^{131}I-Pyrimethamine scintigraphy.

Materials and methods

This study was approved by the local ethics committee of The Experimental Studies at Ege University, School of Medicine. All animals were anesthetized with ketamine 35 mg/kg, intraperitoneal + Xylazin 5 mg/kg during the injection processes. All the reagents were obtained from Merck Chemical Co. Na^{131}I (Monrol, Istanbul, Turkey) was supplied by the Department of Nuclear Medicine of Sifa University. Pyrimethamine was purchased from Sigma (Munich, Germany).

Table 1. Rf values.

	Solvent 1	Solvent 2
^{131}I-Pyrimethamine	0.86	0.89
Na^{131}I	0.58	0.43

Solvent 1: *n*-butanol-ethanol-0.2 N NH_4OH (5-2-1).
Solvent 2: *n*-butanol-water-acetic acid (4-2-1).

Parasites and animal

Five Wistar rats (*Rattus norvegicus*) 90 days old weighing 300–320 g were used. The animals were bred at the premises of the Laboratory of Parasitology Medicine of Ege University. The animals were housed individually in polypropylene cages in a room maintained at 22 ± 1°C, with a controlled 12 h light–dark cycle and 50 ± 10% relative humidity. Feed and tap water were provided *ad libitum*. In this study, the GT F1 strain of *T. gondii* obtained from Ege University, School of Medicine was used. A total of five rats were divided into two groups as the infected and the control group. Three rats of infected group were intraperitoneally inoculated abdomen: with *T. gondii* tachyzoites (750 per rat). At least 2 months earlier were used as sources for tissue cysts. Two healthy rats were in the control group.

Labeling of pyrimethamine with I-131 and quality control

Pyrimethamine was labeled with I-131 using the iodogen method under the same conditions as previously described by Yurt Lambrecht et al. (2009) (Seyitoglu et al., 2009; Yurt Lambrecht et al., 2010). One microgram of Pyrimethamine was dissolved in DMSO and diluted with distilled water (50% vol/vol). Pyrimethamine solution was left in an ultrasonic bath for 30 min at 70°C, and cooled to room temperature. Pyrimethamine solution was added into the iodogen (0.5 mg) coated vial. The coated vial was prepared by evaporating the solvent after 0.5 mg of iodogen was dissolved in dichloromethane (1 mL). Then, Na^{131}I solution (37 MBq/100 µL) was added to the vial. This

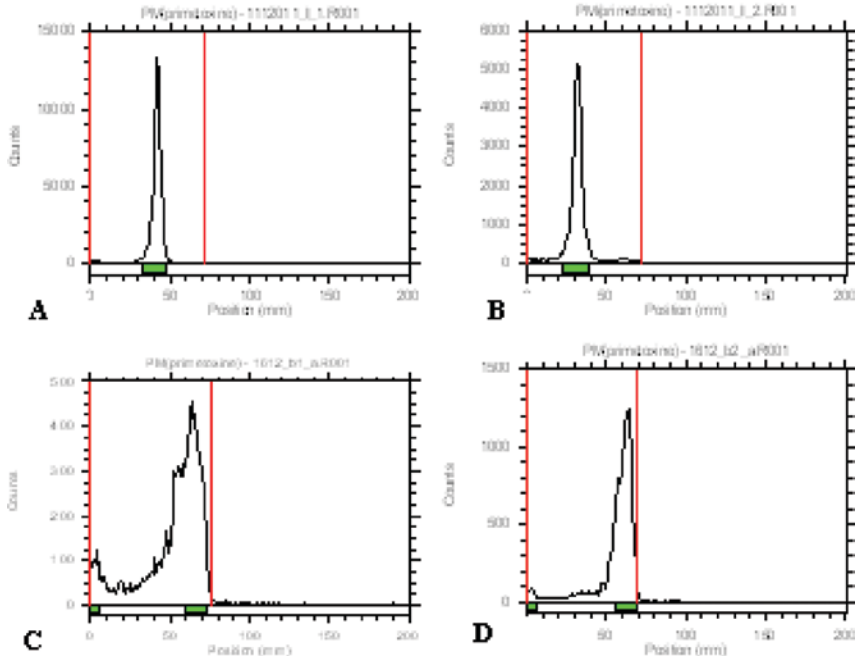


Figure 1. RTLC chromatograms of Na^{131}I (**A**, **B**) and ^{131}I-Pyrimethamine (**C**, **D**) in solvent 1 and 2.

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reaction mixture was kept at room temperature for 30 min. The reaction was ended by adding 100 µL Na_2SO_3 (0.1 N) solution. The radiochemical purity of ^{131}I-Pyrimethamine was determined using radio thin layer chromatography (RTLC) method. The reaction product was spotted on silica gel ITLC-SG strips (10 × 1.5 cm^2 sheets) and developed in solvent 1[*n*-butanol-ethanol- 0.2 N NH_4OH (5-2-1)] and solvent 2 [*n*-butanol-water- acetic acid (4-2-1)]. After developing, the strips were covered with cello-bands. Then, they were scanned with RTLC scanner (Bioscan AR-2000; Bioscan, Washington, DC, USA). And then serum stability of ^{131}I-Pyrimethamine was evaluated up to 24 h.

Scintigraphy

^{131}I-Pyrimethamine (3.7 MBq/rat) was injected via the tail vein to all the rats for imaging studies. Static whole body images of the rats were acquired under the gamma camera at 5 min, 45 min, 2 h, 6 h, and 24 h following the intravenous administration of the radioactivity. Visual and semiquantitative analyses of the images ^{131}I-Pyrimethamine images of all the animals were performed by two independent nuclear medicine specialists (F.Y.L., E.S., R.B.) (matrix: 256x256, zoom: 2, 15 min/per each by using high energy collimator with the forte gamma camera, (Philips, Eindhoven, the Netherlands).

In the visual evaluation, ^{131}I-Pyrimethamine uptake pattern was demonstrated in control Wistar rats (*R. norvegicus*). After that ^{131}I-Pyrimethamine uptake in infected animals was evaluated visually, and the findings were correlated with the control group rats.

Table 2. Serum stability.

Time (h)	Average% yield
1	94.60 ± 0.37
2	93.61 ± 5.53
3	92.05 ± 2.22
4	91.92 ± 1.62
24	80.23 ± 5.19

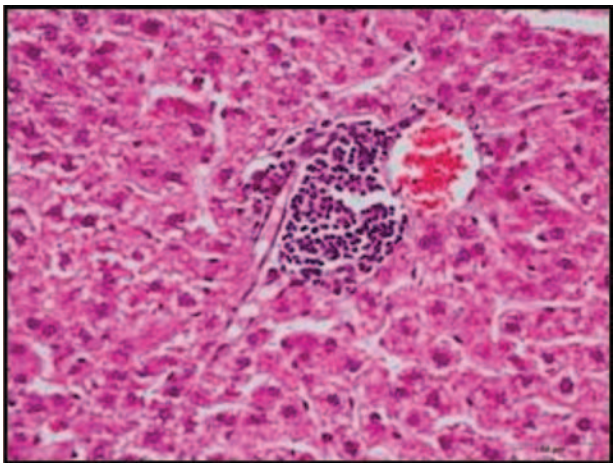


Figure 2. Photomicrographs of rat liver tissues *T. gondii*. Liver sections were stained with haematoxylin/eosin. One of numerous parenchymal nodules of inflammatory cells including mononuclear and polymorphonuclear cells.

The scintigraphic data were analyzed both visually and semiquantitatively. ROIs were drawn over the organs to calculate the ratios of the radiotracer in infected vs. control rats' organs (brain, neck, diaphragm, liver, and total body). Infected/control ratios for organs were calculated using the mean counts at 5 min, 45 min, 2 h, 6 h, and 24 h image to demonstrate ^{131}I-Pyrimethamine in *T.gondii* lesions localized Wistar rats (*R. norvegicus*) body.

Light microscopic examination

Organs of all sacrificed animals were fixed in formalin. Organs were fixed in paraffin for histological examination. Paraffin embedded sections were cut at 5 µm and sections were stained with hematoxylin–eosin (H&E) for microscopic examination (Dubey & Frenkel, 1973).

Statistical analysis

Statistical evaluation was not performed due to the small number of animals in this study.

Results

Labeling procedure and quality control

The labeling efficiency of ^{131}I-Pyrimethamine was determined as to be 96 ± 2 % by RTLC method. In RTLC-SG chromatography using solvent 1, Relative front (Rf) values of Na^{131}I and ^{131}I-Pyrimethamine are 0.86 and 0.58, respectively (Table 1 and Figure 1A, C). Na^{131}I and ^{131}I-Pyrimethamine are 0.89 and 0.43 using solvent 2, respectively (Figure 1B, D). Serum stability of ^{131}I-Pyrimethamine is shown in Table 2. Serum stability as shown by 80% even after 24 h remains stable.

Procedure of histological examination

In order to investigate the cellular components of infected and control rats from different organs, the tissues were processed and stained with haematoxylin/eosin for microscopic examination. The example of such focus in the liver tissue of infected rats is shown (Figure 2). Foci were characterized by the presence of high numbers of infiltrating leukocytes and *T. gondii* bradyzoites.

Results of scintigraphy

^{131}I-Pyrimethamine was accumulated in the thyroid, stomach, liver, bladder, and soft tissue of infected and normal rats (Figure 3). For infected and control group rats, mean infected/control ratios for some organs were given in Table 3. In the visual evaluation, it was noticed that there was increased ^{131}I-Pyrimethamine uptake in the liver of the infected rats according to the normal rats. The uptake by the other organs was similar. The mean ratios of the radiotracer over the liver in infected vs. control were more than 1.0 at 45 min which persisted till 24 h. Similar pattern was seen in the observed ratio in the diaphragm.

Discussion

Rats have been used as an animal model for *T. gondii* infection in humans who also show a high degree of

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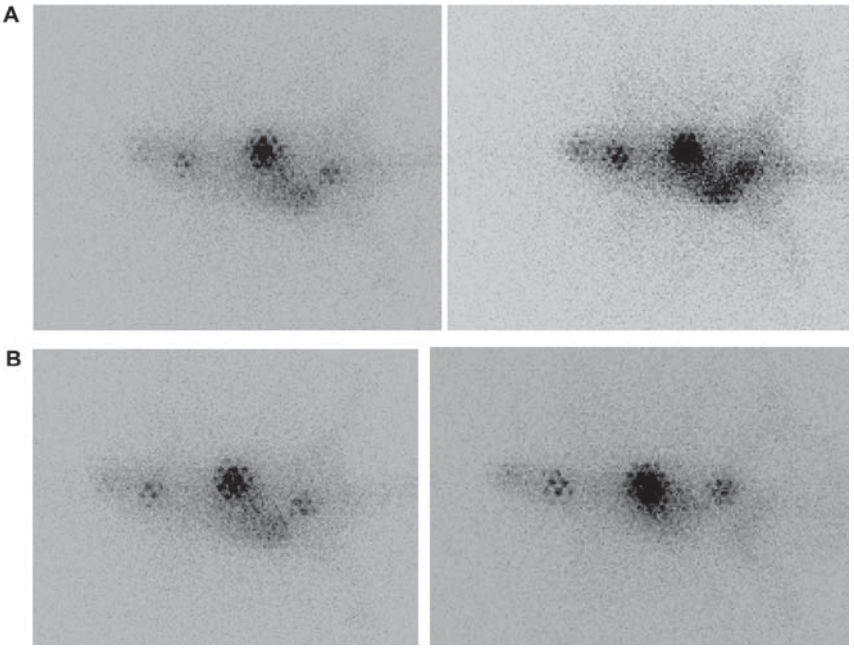


Figure 3. Biodistribution of ^{131}I-Pyrimethamine in rats (**A**) infected and (**B**) normal rats with *Toxoplasma gondii*, at 2th, and 6th-h postinjection Static images of rat showing that increased ^{131}I-Pyrimethamine uptake in the thyroid, stomach, liver, bladder, and soft tissue of infected rats.

Table 3. Infected/control ratios of ^{131}I-Pyrimethamine for the infected and control groups at different time points ($n = 5$).

	Imaging time				
Organs	5 min	45 min	2 h	6 h	24 h
Brain	0.96 ± 0.1	0.92 ± 0.1	0.89 ± 0.1	0.81 ± 0.1	0.87 ± 0.1
Neck	1.15 ± 0.1	0.82 ± 0.1	0.97 ± 0.1	1.60 ± 0.1	1.08 ± 0.1
Diaphragm	0.92 ± 0.1	0.53 ± 0.1	1.00 ± 0.1	1.34 ± 0.1	0.83 ± 0.1
Liver	0.98 ± 0.1	1.64 ± 0.1	1.60 ± 0.1	1.48 ± 0.1	1.20 ± 0.1
Abdomen	0.76 ± 0.1	0.54 ± 0.1	0.62 ± 0.1	0.57 ± 0.1	0.69 ± 0.1

resistance to its pathogenic effects has been known for 50 years (Dubey & Shen 1991; Dubey & Frenkel 1998; Paulino & Vitor 1999).

Nuclear medicine is used for experimental purposes in the diagnosis or the treatment of parasitic infections. It has been previously reported that *Schistosoma mansoni* colitis and *Echinococcus multilocularis* are diagnosed by persistent colonic activity with ^{67}Ga scintigraphy (Chandramouly et al., 1995; Inceboz et al., 2006). In addition, some scintigraphic methods have been used in the diagnosis of different parasitic diseases such as *Fasciola hepatica* (Rivera & Bermudez, 1984), and *Trichinella spiralis* (Inceboz et al., 2012). Gallium-67 uptake in patients with unsuspected cerebral toxoplasmosis and acquired immunodeficiency syndrome (AIDS) was demonstrated in the central nervous system and the lungs by Vanita and Elizabeth (1988). In addition, Ruiz et al. recovered that Thallium-201 might benefit from such investigation to make the distinction between lymphoma and toxoplasma encephalitis in patient with AIDS (Ruiz et al., 1994).

In previous a study, the detection of *T. gondii* in the brain and the muscle tissues was experimentally demonstrated in rats (Freyre et al., 2008). In this experimental study, the uptake of ^{131}I-Pyrimethamine in infected and control animals was evaluated. ^{131}I-Pyrimethamine uptake was seen in the thyroid, stomach, liver, bladder, and soft tissue of infected and normal rats. It was seen slightly increased ^{131}I-Pyrimethamine uptake in the liver of the infected rats according to the normal rats. The mean ratios of the radiotracer over the liver and diaphragm in infected vs. control were >1.0 at 45 min which persisted till 24 h.

Conclusions

In the present study, radioiodine (1–131) was labeled successfully with pyrimethanine. The radioligand was validated by the requisite quality control tests to check its radiolabeling efficiency, *in vitro* stability and radiochemical purity etc. The resultant radiopharmaceutical was evaluated for its diagnostic utility in the scintigraphic detection of experimentally induced *T. gondii* in rats. Radiolabeled I-131-Pyrimethanine (3.7 MBq/rat) was injected intravenously in to the tail vein of the control and infected rats. Static whole body images of the rats were acquired under the gamma camera at 5 min, 45 min, 2 h, 6 h, and 24 h following the intravenous administration of the radioactivity. The scintigraphic data were analyzed both visually and semiquantitatively. ROIs were drawn over the organs to calculate the ratios of the radiotracer in infected vs. control rats. The mean ratios of the radiotracer over the liver in infected vs. control were >1.0 at 45 min which persisted till 24 h. Similar pattern was seen in the observed ratio in the diaphragm. This scintigraphic technique can be a useful imaging modality for the diagnosis of toxoplamosis especially involving liver and diaphragm. The technique however, needs further

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preclinical validation before being extended for use in clinical applications.

Declaration of interest

The authors declared no declaration of interest.

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