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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Technetium-99m labeled Mebendazole and biodistribution in experimentally *Trichinella spiralis*-infected rats

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Abstract The aim of this study was to localize the biodistribution of Technetium-99m (^{99m}Tc) Mebendazole in experimentally *Trichinella spiralis* (*T. spiralis*)-infected rats. Localizing and distinguishing the "*T. spiralis*" in body sites are very important and life saving processes. Scintigraphic detections may help to determine the sites of trichinellosis infection. Twelve healthy female Wistar rats were infected via oral administration of infected muscle containing 750 to 1000 larvae. In this study, the antibiotic was labeled with Tc-99m, and ^{99m}Tc-Mebendazole was assessed as an infection imaging agent in a rat model. ^{99m}Tc-mebendazole was examined for localizing the normal, and inflamed with trichinellosis in rat muscle tissues after administrated ^{99m}Tc-mebendazole via intravenous (IV) or oral gastric catheter, and also for differentiating them from each other. ^{99m}Tc labeled mebendazole was retained in infectious areas. It was established that ^{99m}Tc-mebendazole which was administrated via IV route has high organ restrain level. It was observed that ^{99m}Tc-mebendazole uptake was high in tongue and diaphragm muscles of *T. spiralis*-infected rats after administration of radiolabeled mebendazole via either oral or IV routes. This study may be viewed as a basement of future studies on diagnosis of *T. spiralis* infection.

Keywords *Trichinella spiralis* · ^{99m}Tc-Mebendazole · Infection · Inflammation

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Introduction

Trichinellosis is a worldwide zoonosis caused by nematodes of the genus *Trichinella*. *Trichinella* species occur widely in wild carnivorous animals (Grove, 2000). During its early larval development, *T. spiralis* is an intracellular parasite of mammalian skeletal muscle cells (Jasmer, 1995). Trichinellosis identification of an infection at an early stage of the disease is critical for a favorable outcome. Clinicians use a variety of laboratory, clinical, pathological, and radiological tests to aid diagnosis and make a decision. Although radiological techniques such as X-ray, ultrasound, computerized tomography, and magnetic resonance imaging are commonly used, these techniques are of limited use due to insignificant anatomical changes in the early stages of the infection process (Das *et al.*, 2002; El-Ghany *et al.*, 2005a).

In the world, infection still remains a major cause of mortality and morbidity globally. Because of deep seated

infections can be difficult to detect with variety of laboratory, clinical, and radiological tests.

Nuclear medicine techniques are used due to significant anatomical changes in the early stages of the infection process (Das *et al.*, 2002; El-Ghany et al. 2005a). In this context, in last years, new radiolabeled compounds use the various radionuclide (Ga-67, Tc-99m, In-111, F-18 etc.) labeled small molecules (radiolabeled antimicrobial peptides, antibiotics, antibiotic peptide, and chemotactic peptides) have been developed for infection imaging (El-Ghany *et al.*, 2005b, c; Lupetti *et al.*, 2002, 2003a, b; Sarda *et al.*, 2002; Welling *et al.*, 2000, 1999, 1998; Motaleb, 2007; Hall *et al.*, 1998; Larikka *et al.*, 2002; Pervez and Mushtaq, 2005; Siaens *et al.*, 2004; Yurt Lambrecht 2008a, b, c). Besides, the non-cell labeling agents currently in-used for this purpose are ^{67}Ga-citrate, ^{111}In-IgG, ^{99m}Tc-nanocolloid, ^{99m}Tc-HIG, ^{99m}Tc-BW250/183 antibody etc. ^{99m}Tc-Infecton was a recent agent developed for distinguishing bacterial and sterile inflammation in patients (Sopelas *et al.*, 2003; Roohi *et al.*, 2006; Rennen *et al.*, 2004; El-Ghany et al. 2005b). However, it is also important that trichinellosis infection may also be detected by nuclear medicine technique.

In this study, we used mebendazole which is one of the most effective antihelminthic of the imidazole group and widely used in medicine and veterinary science for the treatment of trichinellosis (Pawlowski, 1981; Fernando and Denham, 1976; McCracken and Taylor, 1980). Anthelmintics are the principal drugs for the treatment of trichinellosis. They include primarily albendazole and mebendazole (Anonymous, 2004).

The aim of this study was to assess the ability to detect the lesions of trichinellosis infection by using ^{99m}Tc-mebendazole biodistribution.

Materials and methods

This study was approved by the local Ethics Committee of The Experimental Studies at Dokuz Eylul University, School of Medicine. All animals were anesthetized with diethyl ether during the injection processes. All the reagents were from Merck Chemical Co. Tc-99m (Monrol, Istanbul Turkey) was supplied by the Department of Nuclear Medicine of Ege University. Mebendazole was purchased from Sigma.

Parasites and animals

The strain of *T. spiralis* GM-1 (ISS048) strain was originally obtained from the laboratory of Parasitology Instituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy and was maintained by serial passage in Wistar rats as previously described (Inceboz *et al.*, 2006a).

Twelve healthy female, 8–12-week-old (approximately 200 g) Wistar rats were chosen for the study, as they were known to be susceptible to *T. spiralis* infection. The study and control groups were also separated equally into two subgroups (oral and intravenous administrated).

Experimental design

Trichinella spiralis larvae were taken from stock infection. Infecting the rats was initiated by oral administration of infected muscle containing 750 to 1000 larvae. After 2–3 months follow-up, chronic infection was established in these infected rats. The *T. spiralis* larvae were sampled, examined histopathologically, and processed to prepare implantation material for the next experiment (Inceboz *et al.*, 2006a).

Radiolabeling procedure

Mebendazole was labeled with Tc-99m via the stannous chloride method under the same conditions as described earlier study by Yurt Lambrecht (2008a, b, c). In the labeling of ^{99m}Tc-mebendazole, mebendazole (0.5 mg) was dissolved with DMSO (0.5 ml) in a vial. pH was adjusted to 3 using 1M HCI. Then, 100 µl $SnCl_2$ solution (1mg/ml) was added to the vial. After addition of all reagents, 37-74 MBq Na $^{99m}TcO_4$ was added into the vial. The vial was incubated at room temperature for 20 min.

Radiochemical analysis of ^{99m}Tc-mebendazole

The labeling yield and radiochemical purity were determined by thin layer chromatography. The reaction product was spotted on silica gel ITLC-SG strips (10×1.5 cm^2 sheets) and developed in 50% triflour acetic acid (TFA) and methyl ethyl ketone (MEK) solution. After developing, the strips were covered with cello-bands. Then, they were scanned with Radio Thin Layer Chromatography (RTLC) scanner (BioScan AR-20).

In RTLC-SG chromatography using 50% TFA as the solvent, R_f values of $^{99m}TcO_4^-$, reduced ^{99m}Tc, and ^{99m}Tc-mebendazole are 0.89, 0.04, and 0.55, respectively. $^{99m}TcO_4^-$, reduced Tc-99m, and ^{99m}Tc-mebendazole are 1.00, 0.0, and 0.95 using MEK solution as solvent, respectively. Radiolabeling yield of ^{99m}Tc-mebendazole was determined as $84.00 \pm 2.00\%$.

Biodistribution studies

Following inflammation process, 23.1–46.2 MBq/µmol/rat ^{99m}Tc-mebendazole at pH = 7 was injected via the tail

vein of control, *T. spiralis*-infected muscle rats. In another group study, ^{99m}Tc labeled mebendazole was administrated via oral gastric catheter to both control and *T. spiralis*-infected rats.

The rats were sacrificed by decapitation under ether anesthesia at 1 h after administrated via IV route and at 2 h after administrated via oral gastric catheter.

Some muscles and organs of both groups of rats were selected, and all of the muscles and organ activities were counted using a Cd (Te) detector equipped with a RAD 501 single channel analyzer. The results were expressed as (%ID/g) the percent uptake of injected dose per gram of organs and muscles.

Histopathology

The preparations, which were prepared from the infected material (muscles), were examined histopathologically under a light microscope, and larvae of *T. spiralis* were identified. In this way, the presence of infection is justified by the gold-standard method.

Statistical analyses

Differences in the mean values of measured activities were analyzed statistically using SPSS statistical software (SPSS for Windows; Release 10.0.1 Standard Version). Comparisons between different groups were performed by Pearson correlation and one-way ANOVA; if ANOVA revealed significant differences, post hoc comparisons were performed by Duncan multiple range tests. $P < 0.05$ was considered significant.

Results

The data related to biodistribution of ^{99m}Tc-mebendazole in *T. spiralis-infected* rats for both administrations were shown in Fig. 1. Figure 1 depicted that the uptake in all organs (%ID/g = 0.37, 0.56, 0.07, 0.16, and 0.39 for liver, spleen, heart, kidneys, and blood, respectively) apart from gaster in administered IV was higher than that in administered oral gastric catheter. When injected oral gastric catheter, the %ID/g value in gaster was 1.88 in infected rats. In both controls, the uptake of organs was higher than those in organs of infected rats. As seen from the Fig. 2, uptakes of the tongue and the diaphragm were high in both groups. When compared both administration the uptake of the diaphragm was higher in *IV*-administrated rats (%ID/g = 0.10) than in infected rats administered via oral gastric catheter (%ID/g = 0.03). In all muscles apart from tongue and *M. semimemranosus*, the uptake of radiolabeled mebendazole was very high in IV-administrated rats comparing with the rats administered via oral gastric catheter.

The uptake in diaphragm (%ID/g = 0.104) was higher five times than control group (%ID/g = 0.030) in *IV*-administrated rats. On the other hand, uptake of ^{99m}Tc-mebendazole in *M. massater* (%ID/g = 0.035) was high two times than control group (%ID/g = 0.017).

Biodistribution of ^{99m}Tc-mebendazole in control group and in *T. spiralis-infected* rats showed different results in rats administered via oral gastric catheter. The uptake was higher in tongue, diaphragm, and *M. massater* than others in *T. spiralis-infected* rats than those in control rats.

Infected and control group of ^{99m}Tc-mebendazole in administered oral gastric catheter showed positive correlation in *m. semitendinosus* masseter ($P = 0.01$), m. intercostalis–

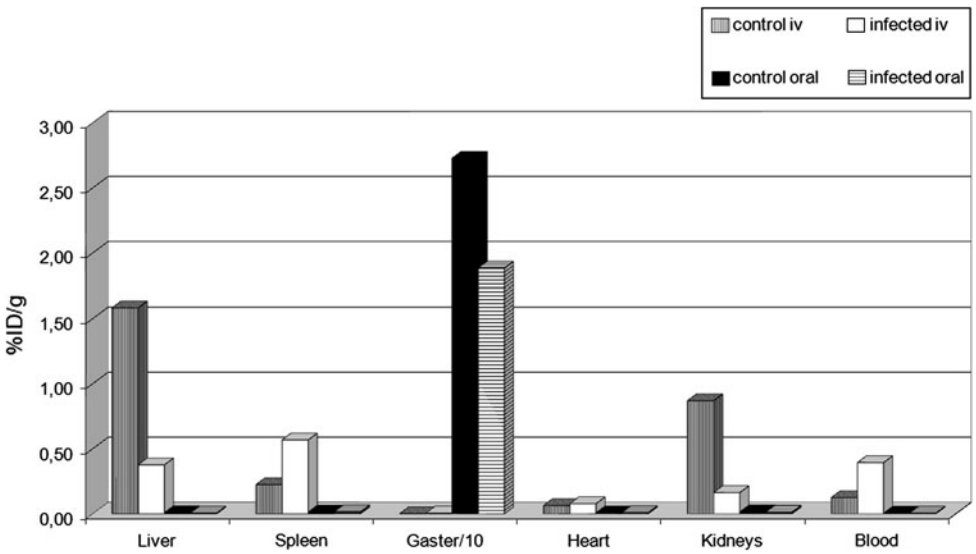


Fig. 1 Biodistribution of ^{99m}Tc-mebendazole in rats (via control, IV, gastro, oral gastric catheter)

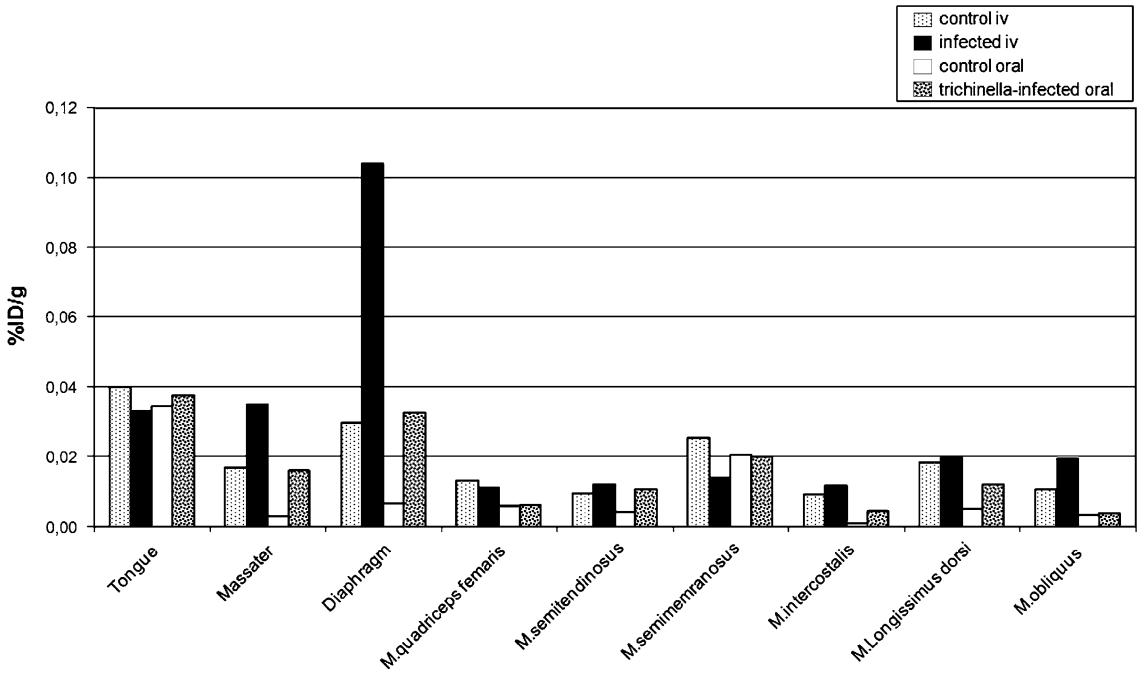


Fig. 2 Uptake of ^{99m}Tc-mebendazole in muscle of rats (via control, IV, gastro, oral gastric catheter)

m. semitendinosus ($P = 0.01$), gaster-m. semitendinosus ($P = 0.03$), spleen–semitendinosus ($P = 0.003$), gaster–heart ($P = 0.024$), spleen–heart ($P = 0.03$), and liver–kidney ($P = 0.02$).

Besides, infected and control group of ^{99m}Tc-mebendazole in IV-administered showed positive correlation in spleen–diaphragm ($P = 0.04$), m. obliquus–m. quadriceps femaris ($P = 0.04$), liver–m. semimemranosus ($P = 0.03$), kidney–m. semimemranosus ($P = 0.02$), kidney–liver ($P = 0.001$).

Discussion

The causative agent of trichinellosis is *T. spiralis* which lives in the intestines in adult form or in skeletal muscles in larval form. After ingestion of raw or undercooked infected meat that contains larvae of *T. spiralis* these larvae enter the blood and the lymphatic circulation. They usually settle in the skeletal muscles and develop further (Inceboz *et al.*, 2006a). The newborn larvae migrate from the intestine of the host to the striated skeletal muscle via blood stream to where they grow and become encapsulated within myofiber and develop into the infective larvae or muscle larvae until another host ingests them. All morphological phases of the parasite develop in the same individual hosts and leads to a zoonosis called trichinosis (Malakauskas *et al.*, 2007; Remonti et al. 2005). There have been some reports demonstrating that mebendazole in experimental trichinellosis (Kotula *et al.*, 1984).

Usually, the early clinical diagnosis of trichinellosis is rather difficult because pathognomonic signs or symptoms are lacking (Dupouy-Camet and Bruschi, 2007), and also, later chronic forms of the disease are not easy to diagnose. In regard specifically to trichinellosis, although several methods have been used for serological diagnosis, ELISA has been and continues the most commonly used method because of its high sensitivity (Gamble *et al.*, 2004). However, there still exists the problem of the cross reaction of sera from persons with disorders other than *Trichinella* sp. For this reason, ELISA is not suitable for epidemiological purposes, especially in areas where other parasites are present (Gamble *et al.*, 2004).

Nuclear medicine imaging techniques are used for experimental purposes in parasitic infection diagnosis or treatment. It has been previously reported that *Schistosoma mansoni* colitis and *Echinococcus multilocularis* are diagnosed by persistent colonic activity on ^{67}Ga scintigraphy. (Chandramouly *et al.*, 1995; Inceboz *et al.*, 2006b). In addition, some scintigraphic methods have been used in the diagnosis of different parasitic diseases such as *Fasciola hepatica* (Rivera and Bermudez, 1984), of *T. spiralis* (Inceboz *et al.*, 2008).

In the study of Inceboz *et al.*, ^{67}Ga scintigraphy was used to detect of *T. spiralis*, after ^{67}Ga was administered, it was waited for the radioactive substance to be held. However, it could not succeed as expected (Inceboz *et al.*, 2008). In our study, mebendazole was labeled with ^{99m}Tc, its potential was investigated for using in imaging of trichinellosis infection. When ^{99m}Tc-mebendazole was

administrated via oral route, the important data were obtained to show the distribution of the drug in the body and when orally administered, as ^{99m}Tc-mebendazole goes directly to stomach, the restrain is very high. Because of this, experimentally the retrain is not significant, administration via IV route would be more useful for determining organ restrain.

In our data, the uptake of radiolabeled mebendazole was high in all muscles of *T. spiralis*-infected rats according to controls in both administrations.

It was established that ^{99m}Tc-mebendazole when administrated IV has high organ restrain level. It was observed that in infected rats, by both administration (either orally or via IV), the restrain of ^{99m}Tc-mebendazole was high in tongue and diaphragm muscles, which were shown to have high *T. spiralis* larvae density in our previous study (Inceboz *et al.*, 2006a).

Conclusion

In conclusion, the results show that ^{99m}Tc-mebendazole has a good uptake in *T. spiralis*-infected muscle. Thus, ^{99m}Tc-mebendazole may be useful for imaging of *T. spiralis-infections.* Future comparative studies with ^{99m}Tc-mebendazole and other infection detecting compounds will further clarify this interesting issue.

Conflict of interest None.

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