

Biodistribution Study of the Anaesthetic Sodium Phenobarbital Labelled with Technetium-99m in Swiss Mice Infected with *Schistosoma mansoni* Sambon, 1907

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Technetium-99m (^{99m}Tc) is a radionuclide that has negligible environmental impact, is easily available, inexpensive and can be used as a radioactive tracer in biological experiences. In order to know the mode of action of sodium phenobarbital in moving adult Schistosoma mansoni worms from mesenteric veins to the liver, we labelled sodium phenobarbital (PBBT) with ^{99m}Tc and a biodistribution study in infected and non-infected Swiss mice was performed. The PBBT was incubated with stannous chloride used as reducing agent and with ^{99m}Tc, as sodium pertechnetate. The radioactivity labelling (%) was determined by paper ascending chromatography performed with acetone (solvent). The ^{99m}Tc-PBBT was administered by intraperitoneal route to Swiss mice infected eight weeks before. The animals were perfused after different periods of time (0,1,2,3,4 hr) when blood, spleen, liver, portal vein, mesenteric veins, stomach, kidneys and adult worms were isolated. The radioactivity present in these samples was counted in a well counter and the percentage was determined. The radioactivity was mainly taken up by the blood, kidney, liver and spleen. No radioactivity was found on the adult worms. We concluded that the worm shift was due to an action on the host of the sodium phenobarbital.

Key words: *Schistosoma mansoni* - technetium-99m-sodium phenobarbital - biodistribution - Swiss mice

An unexpected migration of the adult worm of *Schistosoma mansoni* is observed in albino mice treated with schistosomicides. The worm migrates from mesenteric veins to the liver (Woolhouse & Kaye 1977). This same fact can be observed when these mice are submitted to anaesthetic drugs, as ether and chlorophorm (Khayyal 1965), nembutal (Goldsmith & Kean 1966), fluotane (Motta et al. 1968), halotane (Cunha-Melo & Coelho 1986), pentobarbital and sodium phenobarbital (Machado e Silva et al. 1993, 1996). However, it is not established if this migration is due to the action of the drug in the host and/or directly in the adult worms. The absence of an efficient methodology to perform this study is the reason for this doubt.

The employment of radionuclides in different biological evaluations has permitted a strong tech-

nical-scientific development in various areas of the knowledge. With radioisotopic methodologies, in laboratory conditions, several characteristics of the interaction of the *S. mansoni* with the experimental host have been studied (Christensen 1977, Pinto et al. 1990). Despite the importance of the acquired information using these techniques, they are sometimes restricted, mainly due to (i) the non availability of the radionuclide, (ii) the high costs of the method and (iii) the radioprotection problems (Bernardo-Filho et al. 1992).

Technetium-99m (^{99m}Tc) is a radionuclide that presents several relevant biological, chemical, physical and economical characteristics (Hladik III et al. 1987, Saha 1992, Early & Soddee 1995). It has been widely utilized to label radiopharmaceuticals to be used in nuclear medicine procedures (Bernardo-Filho et al. 1994), as well as radiotracers to be employed in basic research (Bernardo-Filho et al. 1992, Ripoll-Hamer et al. 1995, Santos et al. 1995). In parasitological evaluations, it has been used successfully, as an alternative radionuclide to get information about the *S. mansoni* life

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Received 16 April 1997

Accepted 30 June 1997

cycle. ^{99m}Tc-labeled cercaria did not lose its motility and infectivity, and it is possible to study its migration in albino mice (Bernardo-Filho et al. 1992). The administration of a ^{99m}Tc-labeled oxamniquine solution in infected mice reveals its uptake in specific organs and in the adult worms (Rebello et al. 1994).

In order to evaluate the action mode of sodium phenobarbital to move adult worms (worm shift) from mesenteric veins to the liver, we have labeled sodium phenobarbital with ^{99m}Tc and have studied the biodistribution study of this radiotracer in *S. mansoni* infected swiss mice.

MATERIALS AND METHODS

Experimental infection - Twenty seven males and females outbred mice (seven-day-old) were infected through transcutaneous route with 80 cercariae of *S. mansoni* (BH strain, Brazil).

Labeling study - To establish the optimal stannous chloride (SnCl₂·2H₂O) concentration, 0.5 mg/ml of sodium 5 ethil-5-phenilbarbiturate (PBTT) (1.0 mg/ml), in vacuum tubes, was incubated (10 min, room temperature) with 0.5 ml of various stannous chloride solutions (0.01, 0.1, 1, 5, and 10 mg/ml). Then, ^{99m}Tc (18.5 MBq/ml), as sodium pertechnetate, recently milked from a ^{99m}Tc/⁹⁹Mo generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil) was added and the incubation continued for another 10 min.

Radiochemical control - The percentage of radioactivity (% rad) present in the sodium 5 ethil-5-phenilbarbiturate fraction was determined using strips (8 x 0.8 cm) of Whatman chromatography paper. One drop (2 µl) of ^{99m}Tc-PBBT solution was spotted 1 cm from the bottom of three strips. Solution of 0.9% NaCl and acetone were employed as mobile phases, to test their ability to separate free ^{99m}Tc and ^{99m}Tc-colloid from ^{99m}Tc-PBBT.

Radiochemical stability - Chromatography of the labeled sodium 5 ethil-5-phenilbarbiturate with the best stannous chloride concentration (0.01 mg/ml) was carried out at different times after the labeling reaction to evaluate the labeling stability (1, 2, 3, 4, 5 hr).

Biodistribution study - ^{99m}Tc-PBBT (10 mg/wb) labeled with stannous chloride 0.01 mg/ml was administered, intraperitoneal route, in infected (56 days after the infection) and not infected mice. After a variable period of time has elapsed (0, 1, 2, 3, 4 hr) the animals were sacrificed by cervical displacement and perfused (Machado-Silva et al. 1993). Adult worms, blood, mesenteric vessels, portal vein, stomach, liver, brain, spleen, muscles, intestines, heart and kidneys were isolated. The ra-

dioactivity present in these samples was counted in a well counter. The percentage of radioactivity per gram of tissue was determined for each sample as related elsewhere (Rebello et al. 1994).

Statistical analysis - Analysis of variance (ANOVA) considering p ≤ 0.05 was performed (Remington & Schork 1985).

RESULTS

The study with several stannous chloride concentrations reveals that high labeling yields of the PBBT with ^{99m}Tc are achieved from the concentration of the reducing agent of the 0.01 mg/ml. Free ^{99m}Tc migrates with the mobile phase when the chromatographies were performed with 0.9% NaCl solution or acetone. The ^{99m}Tc-PBBT remains in the origin, independently of the stannous chloride concentration used and the yield of the labeling > 90%.

Table I shows the results obtained from the incubation of a ^{99m}Tc-PBBT in different period of time after the labeled process. This labeled molecule is stable in *in vitro* and the percentage of the radioactivity is not practically modified up to 2 hr, although a slightly reduced is observed after this time.

TABLE I

Evaluation of the stability of the labeled sodium 5 ethil-5-phenilbarbiturate with technetium-99m in different elapsed times after the labeled process.

Acetone was employed as mobile phase of the chromatography. The chromatography was carried out and the percentage of the radioactivity was determined in the bottom and the top of the stationary phase (paper strips)

Time (hr)	Bottom (%)	Top (%)
0	92.8	7.2
1	92.6	7.4
2	92.5	7.5
3	91.5	8.5
4	91.3	8.7
5	90.6	9.4

Table II shows the biodistribution (%) of ^{99m}Tc-PBBT/g in healthy (not infected) mice in different periods of time after the administration of the labeled drug. After 1 hr, the radioactivity is found in the kidney (11.4%). The radioactivity decreased with the time in the spleen from 6.9 (0 hr) to 2.7 (4 hr), in the liver from 5.8 to 3.6% and in the intestines from 5.8 to 4%. It is observed an increasing of the ^{99m}Tc-PBBT in mesenteric system from 3.9 to 5.5% and in portal vein from 6.5 to 13.3% in the same considered times.

Table III shows the biodistribution (%) of ^{99m}Tc -PBBT/g in infected mice in different periods of time after the administration of the labeled drug. The radioactivity uptake in the spleen, in the liver and in the bowels is reduced ($p < 0.05$) when compared with

the healthy animals. However, the fixation of radioactivity is not practically modified in the kidney, in mesenteric system and in portal vein in the same considered times. Radioactivity was not found on the adult worms in the infected mice either.

TABLE II

The biodistribution study of ^{99m}Tc -PBTT in not infected mice. The radioactivity present in different specimens was determined in various periods of time in a well counter. The percentage of radioactivity per gram of tissue was calculated in each specimen

Specimens	Time (hr)				
	0	1	2	3	4
Blood	5.3±0.5	3.0± 0.3	2.5±0.3	2.0±0.1	2.0±0.1
Liver	1.3±0.2	2.5±0.3	2.0±0.2	0.9±0.08	0.7±0.01
Intestines	0.7±0.06	1.8±0.1	0.5±0.04	0.5±0.03	1.2±0.01
Stomach	0.6±0.04	0.7±0.06	1.1±0.1	3.5±0.3	3.4±0.3
Kidneys	2.3±0.2	12.2±1.5	11.2±1.3	6.5±0.5	6.4±0.6
Spleen	0.6±0.06	0.8±0.07	0.3±0.01	0.3±0.02	0.3±0.01
Heart	1.0±0.06	0.8±0.07	0.4±0.03	0.4±0.01	0.3±0.03
Lungs	1.1±0.1	1.2±0.2	1.3±0.1	0.5±0.04	0.6±0.06
Muscle	0.3±0.03	0.8±0.07	0.4±0.03	0.6±0.05	0.2±0.02
Brain	0.0	0.0	0.0	0.0	0.0
Mesenteric	3.3±0.3	4.7±0.5	5.1±0.4	5.6±0.5	6.0±0.5
Portal vein	5.4±0.5	9.0±0.8	9.8±0.9	10.7±1.0	13.9±1.1
Mesenteric worms	-	-	-	-	-
Liver worms	-	-	-	-	-

TABLE III

The biodistribution study of ^{99m}Tc -PBTT in infected mice. The radioactivity present in different specimens was determined in various periods of time in a well counter. The percentage of radioactivity per gram of tissue was calculated in each specimen

Specimens	Time (hr)				
	0	1	2	3	4
Blood	3.3±0.2	2.2±0.2	0.8±0.08	1.6±0.1	1.2±0.1
Liver	5.8±0.5	4.0±0.4	2.7±0.2	2.4±0.3	3.6±0.3
Intestines	5.8±0.5	4.6±0.4	4.0±0.3	3.0±0.2	4.0±0.4
Stomach	3.9±0.4	4.4±0.5	5.7±0.5	3.9±0.3	5.2±0.5
Kidneys	3.4±0.3	11.4±1.0	9.3±0.9	7.8±0.7	6.1±0.5
Spleen	6.9±0.6	5.1±0.5	3.9±0.4	4.1±0.4	2.7±0.3
Heart	0.9±0.08	0.7±0.06	0.6±0.05	0.5±0.05	0.4±0.03
Lungs	1.0±0.1	1.4±0.1	1.0±0.1	0.6±0.07	1.2±0.1
Muscle	0.6±0.06	0.8±0.09	0.6±0.06	0.4±0.01	0.4±0.02
Brain	0.0	0.0	0.0	0.0	0.0
Mesenteric	3.9±0.4	3.1±0.3	3.8±0.3	4.3±0.4	5.5±0.4
Portal vein	6.5±0.6	7.8±0.8	6.2±0.6	7.7±0.7	13.3±1.4
Mesenteric worms	0.0	0.0	0.0	0.0	0.0
Liver worms	0.0	0.0	0.0	0.0	0.0

DISCUSSION

Barbiturate drugs are commonly employed as anaesthetic ones in the surgical routine and in the experimental one in laboratory animals (Zanini & Oga 1982). When these drugs are administered to *S. mansoni* infected mice, a migration of the worms from mesenteric vessels to the liver is observed (Khayyal 1965). Furthermore, this effect is very strong when the anaesthetic is administered by peritoneal route (Machado-Silva et al. 1993) and presents vasodilator action (Machado-Silva et al. 1996). However, this action mechanism of the anaesthetic is not elucidated: if the effect of the drug is direct (i) in the adult worm and/or (ii) in the host. Authors have reported a depressant action on the central ganglion of the worm or their paralysis after a decreasing in their electrical activity (Khayyal 1965, Semejn et al. 1982, Mellin et al. 1983).

The quantification of radioactivity in adult worms of the *S. mansoni* has been possible. The methodology using radionuclide has been adequate to evaluate the fixation of ^{99m}Tc-oxamniquine into adult worms (Rebello et al. 1994).

Our results indicate that the phenobarbital seems to have an indirect action in the adult worms due to the radioactivity of ^{99m}Tc-labeled drug was not found in the worms (Table III). The biodistribution study of the ^{99m}Tc-PBBT reveals the presence of an important amount of radioactivity in the liver, portal vein, mesenteric vessels, spleen, intestines and kidneys.

A decreasing in the blood flowing, an increasing in the blood pressure and in the vessel lumen have been described after the administration of sodium phenobarbital (Machado-Silva et al. 1996).

Hepatic and renal vessels have β -adrenergic receptors when these receptors are stimulated, they induce vasodilatation (Zanini & Oga 1982). They probably are binding sites to the sodium phenobarbital due to this drug is a vasodilator one.

The biodistribution of radiopharmaceuticals can be altered due to various factors as drug interaction, radiation therapy or diseases (Goodman & Gilman 1991). The anatomical, histological and physiopathological alterations due to the schistosomiasis infection could justify the decreasing of the radioactivity uptake in liver and spleen (Coelho et al. 1977). In the *S. mansoni* infected animals there is a severe diminishment of the hepatic function and its capacity to metabolize drugs is reduced due to the microsomal enzyme activities are altered (Cha et al. 1980).

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