Long-Term Tissue Distribution and Steady State Activity Ratios of ^{232}Th and Its Daughters in Rats after Intravascular Injection of Thorotrast

Toshiyuki NORIMURA¹, Takehiko TSUCHIYA¹, Satoru HATAKEYAMA²,
Hisao YAMAMOTO² and Shunzo OKAJIMA³

¹*Department of Radiation Biology and Health, School of Medicine, University of Occupational and Environmental Health, Japan. Kitakyushu 807, Japan*

²*Radioisotope Research Center, University of Occupational and Environmental Health, Japan. Kitakyushu 807, Japan*

³*Atomic Disease Institute, Nagasaki University School of Medicine. Nagasaki 852, Japan*

Abstract : To estimate the absorbed dose in the critical organs of Thorotrast patients, it is necessary to know not only the distribution and concentration of ^{232}Th but also its daughter nuclides in the body. The present investigation was undertaken in order to clarify the long-term ^{232}Th tissue distribution and steady state activity ratios between subsequent daughters in the critical tissues using about 30 Wister male rats, as a basis for estimating absorbed doses. The tissue distribution of thorium was examined by means of an autoradiography of the whole body and/or the gamma-ray spectrometry at various times during 2 to 24 months following injection. The concentrations of daughter nuclides in tissues were determined by repetitive gamma examination over a period from 1 hr to 35 days after being sacrificed. The data indicate (1) that approximately 90% of injected Thorotrast is retained in the body for a prolonged period, but about 50% of radium and 10% of radon produced from thorium are eliminated from the body, (2) that the mean steady state activity ratios of ^{224}Ra and ^{212}Pb to ^{228}Th for liver are 0.56 and 0.28, and 0.54 and 0.16 for spleen, 0.58 and 0.82 for lungs, respectively, and (3) that the parent ^{228}Th is translocated to the bone.

Key words : Thorotrast, body burden, tissue distribution, steady state activity ratio, rats.

(Received 15 May 1989, accepted 16 June 1989)

Introduction

Thorotrast, a 24 to 26 percent colloidal solution of thorium dioxide, had been widely used in many countries as a radiological contrast medium up until about 1950, at which time there was a growing recognition of its potential toxicity. In Japan, it was used mostly at military hospitals for angiography and hepatolienography of wounded soldiers up until 1945. Since then its use has been limited to laboratory studies of its toxicity. Once injected into the circulatory system, almost all the thorium is retained in the body indefinitely and form aggregates. The radiation from these aggregates continues to irradiate tissues for a long time. Actually, many cases of fatal late effects that are considered to be due to Thorotrast radiation have been reported (Faber, 1979; Kaick *et al.*, 1983; Rubel & Ishak, 1982; Mole, 1979; Motta *et al.*, 1979; Mori *et al.*, 1983; Stover, 1983; Wegener & Wesch,

1979), and its carcinogenic effect has been proven in many animal experiments (Okamoto, 1974; Wegener *et al.*, 1983; Wesch *et al.*, 1983). However, the dose-effect relationship between Thorotrast radiation and its influence has not been satisfactorily understood yet. The major reason for this is probably related to the incomplete estimation of the absorbed dose in the organs of Thorotrast patients as well as in animals.

There is a very complicated problem involved in the Thorotrast radiation dosimetry. This complexity is, in part, due to the different behavior of ^{232}Th and its decay products in the body. Thorium, the major ingredient of Thorotrast, possesses a decay series consisting of 11 radionuclides of 8 different elements with half-lives ranging from 0.3 μsec to 1.4×10^{10} yr as shown in Table 1. Each of the elements which comprise this series will exhibit its own characteristic metabolic behavior and movement within the body. Therefore, it is necessary to know not only the distribution and concentration of ^{232}Th but also of its daughters throughout the body.

The liver, spleen, lungs and bone are principal sites of deposition and long-term retention for thorium dioxide injected into the circulatory system (Kato, 1968; Kaul & Noffz, 1978; Norimura, 1977). The present investigation was undertaken in order to confirm the long-term distribution and metabolic behavior of ^{232}Th and its daughters in these critical organs in Wister rats after Thorotrast was intravenously injected, as a basis for estimating absorbed doses.

Table 1. Physical properties of the thorium decay series with a partial listing of gamma energies

Nuclide	Half-life	Decay constant (per year)	Alpha-ray energy MeV intensity (%)		Gamma-ray energy MeV intensity (% of ^{232}Th decay)
^{232}Th	1.4×10^{10} yr	0.495×10^{-10}	4.010	77	Ra L X-rays
			3.952	23	
^{228}Ra	5.75 yr	0.120	—	—	—
^{228}Ac	6.13 hr	0.990×10^3	—	—	0.909 (25), 0.967 (20%, complex)
^{228}Th	1.913 yr	0.362	5.424	71	0.084 (1.2)
			5.341	28	
			5.212	0.5	
^{224}Ra	3.64 day	0.695×10^2	5.684	94.8	0.241 (3.7)
			5.447	5.2	
^{220}Rn	55.3 sec	0.395×10^6	6.228	100	—
^{216}Po	0.15 sec	0.146×10^9	6.777	100	—
^{212}Pb	10.64 hr	0.571×10^3	—	—	0.239 (77), 0.301 (3.2)
^{212}Bi	60.60 min	0.601×10^4	6.050	25.2	0.727 (7)
			6.089	9.8	
			5.767	0.62	
			5.606	7.40	
^{212}Po	0.30 μsec	0.728×10^{14}	8.785	64.07	—
^{208}Tl	0.07 min	0.119×10^6	—	—	2.614 (35.9), 0.583 (29)

Materials and Methods

Animals:

Thirty male Wister rats were obtained at six weeks of age from Funabashi Experimental Animal Company Ltd. (Funabashi, Japan) and observed for five weeks during which they were checked for body weight and infections. All rats were placed in individual mesh wire cages and maintained in a $24 \pm 2^\circ\text{C}$ air-conditioned room and fed commercial pellet chow and tap water *ad libitum*.

Thorotrast injection:

The rats were singly injected intravenously with 0.5 to 2 ml of Thorotrast (Testagar and Co., Inc., USA) at 11 weeks of age (weight about 300 g). These rats were sacrificed at various times over a period of 2 to 24 months following injection and used for *in vitro* analytical measurements.

In vivo measurement:

The whole body retention of ^{232}Th and its daughters was measured in a few rats by repetitive direct *in vivo* gamma counting of the rats for a period of over 100 days after injection using a semiconductor radiation detector (60 ml, Pure Germanium Detector). Only three of the radionuclides of the thorium decay series, ^{228}Ac , ^{212}Pb and ^{208}Tl emit sufficiently distinctive gamma radiations for identification and measurement. The ^{228}Ra and ^{228}Ac were determined from a 0.91 MeV photopeak of ^{228}Ac , and the ^{228}Th , ^{224}Ra and ^{212}Pb were determined from a 2.61 MeV photopeak of ^{208}Tl and/or a 0.24 MeV photopeak of ^{212}Pb .

Whole body autoradiography:

The distribution of ^{232}Th and its daughters in the rats was examined by means of a whole body autoradiography. The rats were sacrificed 2, 5, 8 and 12 months after injection and immediately chilled in acetone dry ice (-78°C). After gently raising the temperature, the rats were sectioned at -20°C to make freeze-dried whole body sections of 30 μm thick. X-ray film (Fuji X-ray Film, 150) was attached to the freeze-dried sections and they were left exposed for about 3 months.

In vitro measurement:

The rats were sacrificed at the same time as the foregoing whole body autoradiography and such critical tissues as liver, spleen, lungs, bone, etc., were removed and used for analysis. Tissue samples were immediately sealed in a polyethylene container and the activity of the samples was analyzed by gamma-ray spectrometry using the Ge semiconductor detector at various times until ^{228}Th reached radioactive equilibrium with its daughters. A growth of an activity ratio of ^{212}Pb to ^{228}Th , $(\lambda_{8\text{N}_8}/\lambda_{4\text{N}_4})_{\text{T}}$, was obtained from the 0.24 MeV photopeak of ^{212}Pb and given by the following formula:

$$\left(\frac{\lambda_8 N_8}{\lambda_4 N_4}\right)_T = 1 - 1.139(1 - \alpha_5) \cdot e^{-0.189t} + (0.139 - 1.139 \cdot \alpha_5 + \alpha_8) \cdot e^{-1.563t}, \quad (1)$$

where t is the time after death, and α_5 and α_8 are the activity ratio of ^{224}Ra to ^{228}Th and ^{212}Pb to ^{228}Th in tissues, respectively, just before sacrifice ($t=0$).

Results

Whole body retention of ^{232}Th and daughter nuclides:

The whole body retention curves of the three radionuclides obtained from six animals are shown in Fig. 1. ^{228}Ac , a daughter nuclide of ^{232}Th was reduced to 50% of the injected

Fig. 1. Body burdens of ^{228}Ac , ^{212}Pb and ^{208}Tl in rats as a function of time after Thorotrast injection. Average value of 6 animals. Standard deviations are $\pm$ about 10%.
 ● ^{212}Pb ○ ^{208}Tl ■ ^{228}Ac

Fig. 2. Long-term whole body retention and growth curves of activity of daughter nuclides after sacrifice. Average value of 3 animals.
 ● 0.1–4 MeV ○ ^{212}Pb

Fig. 3. Whole body autoradiographs of Thorotrast in a rat at 5 months after injection. A high concentration of radioactivity is seen in the spleen and lymph node, and a deposit of thorium in the liver and cortical bone is also observable.

amount during 2 weeks after injection, and ^{212}Pb and ^{208}Tl was reduced to 45% after 4 days, and then reached a steady state. When these rats were sacrificed under the steady state conditions, however, the activity of the daughter nuclides, such as ^{224}Ra and lower ones, e. g., ^{212}Pb and ^{208}Tl again increased and recovered to about 90% of the injected amount (with correction of radioactive decay) 2 weeks after being sacrificed (Fig. 2). This indicates that the injected Thorotrast was retained in the body for a prolonged period while undergoing little elimination, whereas such daughter nuclides as radium and radon were primarily eliminated from the body, as seen by the growth curve in Fig. 2.

Tissue distribution of ^{232}Th and its daughters:

1) Whole body autoradiography

Figure 3 shows the whole body autoradiographs 5 months after injection. The upper area shows one obtained from a frozen slice of the left-side parallel section of a rat, while the lower area shows one obtained from a frozen slice of the midline section. As can be clearly seen from the autoradiographs, the highest photographic density is exhibited at the spleen and lymph node, and a deposit of thorium in the liver and cortical bone is also observable. There is no marked density in the bone marrow, and in view of the results of gamma-ray spectrometry mentioned below, the density of cortical bone is thought to be due to the uptake of radium. All of the results of the whole body autoradiographs 2, 8 and 12 months after injection led to the observation that the highest density was at the spleen and lymph nodes, the liver and cortical bone follow this, and the lowest density was seen in the lungs (Table 2). Since these autoradiograph results do not quantify the distribution of ^{232}Th and its daughters in the body, we measured their activity in the principal organs (excluding lymph node), in which high density was found, as well as in the blood.

Table 2. Relative concentrations of ^{228}Th in tissues as a function of time after intravenous injection of Thorotrast in Wister male rats

Tissue	by Autoradiography ^{a)}				by Gamma spectrometry ^{b)}			
	2	5	8	12 ^{c)}	2	5	8	12 ^{c)}
Liver	≡	≡	≡	≡	1	1	1	1
Spleen	≡	≡	≡	≡	4.7	5.6	9.4	9.5
Lung	±	±	+	+	0.1	0.2	0.2	0.4
Cortical bone	±	+	≡	≡	0.1	0.4	0.5	0.9
Lymph nodes	≡	≡	≡	≡				
Blood					<0	<0	<0	<0

^{a)} Photographic density.^{b)} The relative radionuclide concentrations were calculated as time of death, by normalizing Liver to = 1.^{c)} Time after injection (months).

2) Gamma-ray measurement

From a series of gamma spectrometry measurements on a tissue at various intervals beginning immediately following the death of the animals, the concentrations of ^{228}Ra , ^{228}Ac , ^{228}Th , ^{224}Ra and ^{212}Pb in the tissue at the time of death may be calculated. Since ^{228}Th reaches radioactive equilibrium with its daughters within one month after being sacrificed, the activity of ^{212}Pb thereafter becomes equal to that of ^{228}Th in the tissue at the time of death. The activity ratios of ^{224}Ra and ^{212}Pb to ^{228}Th in the tissue at the time of the death of the rats can be calculated from the growth curves of the ^{212}Pb obtained by the least-squares solution by being substituted into equation (1). The result of a typical experiment is shown in Fig. 4. Details on the method of calculation have been described by Norimura (1977).

Fig. 4. Growth curves of ^{212}Pb activity in the liver, spleen, lungs and bone of rats 5 months after injection as a function of time after sacrifice.

—●— Liver —○— Spleen —●— Lung —○— Bone

Table 3. Relative concentrations of ^{228}Ac and ^{212}Pb in tissues as a function of time after intravenous injection of Thorotrast in Wister male rats

Tissue	$^{228}\text{Ac}^{\text{a)}}$				$^{212}\text{Pb}^{\text{a)}}$			
	2	5	8	12 ^{b)}	2	5	8	12 ^{b)}
Liver	1	1	1	1	1	1	1	1
Spleen	4.6	4.4	9.1	9.3	3.4	3.7	5.7	5.4
Lung	0.2	0.3	0.3	0.5	0.5	0.6	0.8	1.1
Bone	3.1	4.9	4.5	5.9	1.7	2.1	2.2	3.1
Blood	0.01	0.01	<0	<0	0.7	0.8	0.8	0.5

^{a)} The relative radionuclide concentrations were calculated as time of death, by normalizing Liver to = 1.

^{b)} Time after injection (months).

Regarding the distribution of ^{228}Th 2 months after injection, 60% of the total deposit in the body was taken up by the liver and 14 to 25% by the spleen. Table 2 shows a change in time series in the ^{228}Th concentrations in the principal tissues by normalizing the liver to = 1. The concentration of ^{228}Th in the spleen increased over time after injecting Thorotrast, reaching 9.5 times of the concentration in the liver 12 months after injection, and it also increased in the bone and lungs. Table 3 shows the time-series changes in the concentrations of ^{228}Ac and ^{212}Pb in the principal tissues at the time of death using liver concentration as the standard. In bone, the concentrations of these radionuclides increased in time series: such increase is probably due to the uptake of radium. As regards to blood, at any time (2 to 12 months after injection) the activity of ^{212}Pb decreased rapidly immediately after sacrificing and decreased to 22% one day after sacrifice. This suggests the presence of little ^{228}Th in the blood.

Discussion

After intravascular injection, thorium dioxide aggregates are accumulated in the reticuloendothelial system and are retained for a long time in these tissues; its biological half-life is said to be over 200 years (ICRP, 1968). Kaull & Noffz (1978) reported that the mean organ distribution of ^{232}Th in a standard patient after intravascular injection of Thorotrast can be estimated as follows: liver 59%, spleen 29%, red bone marrow 9%, calcified bone 2.4%, lungs 0.7% and kidneys 0.1%. Thus, the liver and the spleen in particular could be subject to continuous internal exposure for a prolonged period, causing a high risk of malignant tumors due to radiation.

The estimation of the absorbed dose in the critical organs of the Thorotrast patients is very complicated, unlike the case of a single radionuclide, because of the peculiarities of the ^{232}Th , that is, the major ingredient of Thorotrast. About 90% of the energy imparted to tissues by radiation from thorium dioxide is carried by alpha rays (Kato, 1967), and most of it is imparted by daughter nuclides of the thorium. Accordingly, it is essential for estimating the absorbed dose to clarify the metabolic behavior of these daughter nuclides in the body.

The change in process over time in the activity of all gamma rays released in the body of a Thorotrast-injected rat indicates a double-phase curve including a first component in which the activity rapidly decreases to 68% of that at the time of one day after injection and the other which is a steady state condition observable after 14 days. It is thought that the activity in the first component is not due to the elimination of colloidal thorium dioxide itself, but is the result of the daughter nuclides of thorium being excreted outside the body until colloidal thorium dioxide is deposited in the tissues. The major nuclides excreted outside the body are radium because the activity of gamma rays after sacrifice recovers up to about 90% of that at the time of injection, and further because the growth curve of activity is related to the decay constant of ^{224}Ra . In addition, the activity increase after sacrifice also signifies that in the second phase, which is in the steady state, the state of dynamic equilibrium has been established between the amount of daughter nuclides produced from thorium deposited in the tissues and the amount of nuclides which is excreted from the body. While in this case the major daughter nuclides excreted from the body are likewise radium, about 10% of radon is further presumed to have been excreted through respiration by basing the calculation on the fact that the activity of ^{228}Ac is higher than that of ^{212}Pb under steady state condition. These results are in agreement with the work of Hursh

Fig. 5. Activity ratio of ^{224}Ra to ^{228}Th in the critical tissues as a function of time after Thorotrast injection. Values are means $\pm$ standard deviation.

●— Liver ○--- Spleen ▲--- Lung △--- Bone

(1965), who injected Thorotrast into the body of four volunteers and measured ^{220}Rn in exhalation, reporting that about 9% of ^{220}Rn produced was excreted in exhalation.

Figures 5 and 6 show that time-series changes in the steady state activity ratio of ^{224}Ra and ^{212}Pb to ^{228}Th in such critical organs as the liver and spleen has been achieved. The steady state activity ratios started to be stabilized from around 8 months after the injection of Thorotrast, leading to no major change over time in most tissues, excepting bone. The data for bone indicates that the activity ratios between ^{228}Th and its daughters decrease over time after injection. This is thought to be due to the progressive accumulation of ^{228}Th , a parent nuclide. The mean values of the activity ratio of ^{224}Ra and ^{212}Pb to ^{228}Th obtained from the data after 8, 12 and 24 months of injection were 0.56 and 0.28 for the liver, and 0.54 and 0.16 for spleen, 0.58 and 0.82 for lungs, respectively.

In conclusion, the data presented here regarding the long-term distribution and metabolic behavior of ^{232}Th and its daughters in the critical tissues are generally consistent with those of similar investigations by others. However, the values of the steady state activity ratio of ^{224}Ra to ^{228}Th and, in particular, on the ^{212}Pb to ^{228}Th are 20 to 40% smaller than those of Kato (1968) and Kaul & Noffz (1978), who estimated the activity ratios between thorium decay products in tissues using autopsy samples from Thorotrast patients. In

Fig. 6. Activity ratio of ^{212}Pb to ^{228}Th in the critical tissues as a function of time after Thorotrast injection. Values are means $\pm$ standard deviation.

●— Liver ○--- Spleen ▲— Lung △--- Bone

part, this discrepancy is probably related to the difference in the evaluation of time after death, which is most essential to estimate the steady state activity ratios between the short-lived thorium daughters in tissues. Since it is difficult to get fresh tissue samples from Thorotrast patients immediately after death, our present data seems to give more information concerning the metabolic behavior of ^{232}Th and its daughters in the critical tissues that estimate absorbed doses in Thorotrast patients. In addition, we note that the result presented in the whole body autoradiographs reemphasize a need for a careful consideration of the contribution and/or the influence of gamma radiations that result from the decay of ^{228}Ac , ^{212}Pb and ^{208}Tl in the lymph nodes and cortical bone on the determination of the amounts and distribution of thorium in human subjects *in vivo* by the gamma-ray spectrometry using a whole-body counter.

Acknowledgment

This research was supported in part by Grants-in-Aid for Cancer and Scientific Research from the Ministry of Health and Welfare, Japan.

References

- Faber, M. (1979): Twenty-eight years of continuous follow-up of patients injected with Thorotrast for cerebral angiography. *Environ. Res.*, 18: 37–43.
- Hursh, J. B. (1965): Body content of thorium 232 daughters after Thorotrast injection. *Br. J. Radiol.*, 38: 776–784.
- International Commission on Radiological Protection (ICRP) (1968): Radiation protection. ICRP Publication 10. Pergamon Press, Oxford.
- Kaick, G. V., Lieberman, D., Lorenz, D. *et al.* (1983): Recent results of the German Thorotrast study — Epidemiological results and dose effect relationships in Thorotrast patients. *Health phys.*, 44 (Suppl. 1): 299–306.
- Kato, Y. (1967): Alpha-ray dosage near Thorotrast aggregate. *Nippon Acta Radiol.*, 26: 1547–1556.
- Kato, Y. (1968): Determination of thorium amount in Thorotrast patients. *Nippon Acta Radiol.*, 28: 217–228.
- Kaul, A. & Noffz, W. (1978): Tissue dose in Thorotrast patients. *Health Phys.*, 35: 113–121.
- Mole, R. H. (1979): Carcinogenesis by Thorotrast and other sources of irradiation, especially other α -emitter. *Environ. Res.*, 18: 192–215.
- Mori, T., Kato, Y., Kumatori, T. *et al.* (1983): Epidemiological follow-up study of Japanese Thorotrast cases-1980. *Health Phys.*, 44 (Suppl. 1): 261–272.
- Motta da, L. C., Silva Horta da, J. & Tavares, M. H. (1979): Prospective epidemiological study of Thorotrast-exposed patients in Portugal. *Environ. Res.*, 18: 152–172.
- Norimura, T. (1977): Experimental studies on estimation of absorbed dose of critical organ for ThO_2 . *Nagasaki Med. J.*, 52: 25–38.
- Okamoto, T. (1974): Hepatic cholangiocarcinoma in Syrian hamster induced by Thorotrast administration. *Nippon Acta Radiol.*, 34: 254–266.
- Rubel, L. R. & Ishak, K. G. (1982): Thorotrast-associated cholangiocarcinoma. *Cancer*, 50: 1408–1415.
- Stover, B. J. (1983): Effects of Thorotrast in humans. *Health Phys.*, 44 (Suppl. 1): 253–257.
- Wegener, K. & Wesch, H. (1979): Pathological changes of lymph nodes in Thorotrast patients. *Environ. Res.*, 18: 245–255.

- Wegener, K., Hasenohrl, K. & Wesch, H. (1983): Recent results of the German Thorotrast study—Pathoanatomical changes in animal experiments and comparison to human Thorotrastosis. *Health Phys.*, 44 (Suppl. 1): 307–316.
- Wesch, H., Kaul, A., Wegener, K. *et al.* (1983): Recent results of the German Thorotrast study—Statistical evaluation of animal experiments with regard to the nonradiation effects in human Thorotrastosis. *Health Phys.*, 44 (Suppl. 1): 317–321.

トロトラスト静脈注入ラットにおける ^{232}Th とその娘核種の長期体内分布と崩壊率比

法村 俊之¹・土屋 武彦¹・畠山 智²・山本 久夫²・岡島 俊三³

¹産業医科大学放射線衛生学教室 ²産業医科大学アイソトープ研究センター

³長崎大学医学部原爆後障害医療研究施設

要 旨: トロトラスト患者の臓器吸収線量を推定するには、その主成分である ^{232}Th の体内分布量は勿論、放射性崩壊によって生じる各娘核種の崩壊率比を知る必要がある。ラット尾静脈より注入されたトロトラストは、その約 90% が生体内に沈着し長期間残留する。しかし、娘核種のうち Ra の約 50%, Rn の 10% が常時体外へ排泄されている。この動的平衡時における肝・脾臓での崩壊率比は、 $^{224}\text{Ra}/^{228}\text{Th}$ がそれぞれ 0.56, 0.54, $^{212}\text{Pb}/^{228}\text{Th}$ が 0.28, 0.16 であり、これまでに報告されたトロトラスト患者の剖検組織標本の測定による推定値に比し、20–40% 低い値を示した。特に短寿命娘核種の崩壊率比の推定には死亡直後からの経時的な γ 線計測が不可欠である。さらに、リンパ節に顕著な沈着が認められ、骨では Ra の持続的取り込みによる ^{228}Th の蓄積が確認された。体外 γ 線計測による吸収線量推定には、これらの影響をも考慮した評価が必要である。

J. UOEH (産業医大誌), 11 (3): 287–297 (1989)