Research article

**Sodium pertechnetate (Na99mTcO4) biodistribution in mice exposed to cigarette smoke**

Samuel S Valenca*1, Elaine AC Lima2, Gláucio F Dire2, Mário Bernardo-Filho2 and Luís Cristóvão Porto1

Address: 1Departamento de Histologia e Embriologia, Universidade do Estado do Rio de Janeiro, Av. Prof. Manoel de Abreu 444 3° andar – Rio de Janeiro, RJ – 20551-170 Brasil and 2Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro, Av. Prof. Manoel de Abreu 444 3° andar – Rio de Janeiro, RJ – 20551-170 Brasil

Email: Samuel S Valenca* - valenc@zipmail.com.br; Elaine AC Lima - elaine-alves@uol.com.br; Gláucio F Dire - gdire@ubbi.com.br; Mário Bernardo-Filho - bernardo@uerj.br; Luís Cristóvão Porto - lcporto@uerj.br

* Corresponding author

**Abstract**

**Background:** The biological effects of cigarette smoke are not fully known. To improve our understanding of the action of various chemical agents, we investigated the biodistribution of sodium pertechnetate (Na99mTcO4) in mice exposed to cigarette smoke.

**Methods:** Fifteen BALB/c male mice were exposed to the smoke of nine whole commercial cigarettes per day, 3 times/day, for up to 10 days to whole body exposure in a chamber. A control group of 5 BALB/c male mice was sham-smoked. One day later, the exposed and control groups of mice received (7.4 MBq/0.3 ml) of Na99mTcO4 before being killed at 30 min. Bones, brain, heart, intestine, kidney, liver, lungs, muscle, pancreas, spleen, stomach, testis and thyroid were weighed and these organs and blood radioactivity recorded with a gamma counter. The percentage per gram of tissue of injected dose (%ID/g) was determined for each organ.

**Results:** Cigarette smoke significantly decreased (p < 0.05) the %ID/g in red blood cells, bone, kidney, lung, spleen, stomach, testis and thyroid of the exposed mice.

**Conclusion:** The toxic effects of cigarette smoke reduced the Na99mTcO4 biodistribution.

**Background**

Tobacco can be smoked in cigarettes, cigars, pipe, water pipes, or chewed. It is as an important cash crop, and has vast economical impact affecting the livelihoods of the farmers growing it, the companies that manufacture it, and the healthcare system that deals with the consequences of using it. Cigarette smoking (CS), the most popular method of smoking tobacco, is one of the most prevalent social habits practiced worldwide today [1]. The World Health Organization estimated that almost 1.1 billion people are smokers. Smoking has been identified as the leading preventable cause of death and disability in the world [2,3].

The development of diagnostic tools using radioisotopes is widely used in almost all hospitals nationwide. In nuclear medicine practice, physicians set norms for morphological or physiological function for each organ by diagnosing a large number of patients. For every procedure, there is diagnostic data for a range of normal
variations familiar to physicians. Nuclear medicine practitioners interpret diseases on the basis of deviations from these limits [4]. Radioisotopes provide vital information to help diagnosis and therapy of various medical diseases. Data on tissue shape, function and localization within the body are relayed by use of one of various radionuclides, which can either be a free chemical species or covalently bound to part of a larger organic or inorganic moiety. These images are generated by the distribution of radioactive decay of the nuclide [5]. Technetium-99m ($^{99m}$Tc) is the most frequently used radionuclide in diagnostic nuclear medicine procedures for a wide variety of diseases [6]. Various radiochemicals have been labelled with this nuclide. $^{99m}$Tc has a half-life of 6 h and emits γ-rays of 140 keV with an abundance of 90%. $^{99m}$Tc is primarily obtained from $^{99}$Mo/$^{99m}$Tc generator and is eluted as sodium pertechnetate (Na$^{99m}$TcO$_4$). In this chemical form, it can be used to study brain and thyroid [7]. Intravenously administered $^{99m}$Tc-pertechnetate is loosely bound to plasma proteins and rapidly moves out of the intravascular compartment. The plasma half-time clearance is $\approx$30 min. Approximately 30% of the administered dose is excreted within 24 h. The total urinary and faecal excretion of $^{99m}$Tc activity is about 50% in 3 days and up to 70% after 8 days. $^{99m}$Tc is also trapped by the thyroid gland and it passes into the small intestine. However in the brain, the blood-brain barrier prevents Na$^{99m}$TcO$_4$ from entering brain cells [6,7].

The biodistribution and kinetics of radiochemicals can be altered by a variety of chemical agents, as is widely known [8,9]. Without knowing that these chemical agents are being used, the images can be affected to give poor organ visualization, possibly necessitating repeating the procedure, and resulting in unnecessary irradiation or even misdiagnosis [10]. The effect of CS on the biodistribution of radiochemicals has not been fully evaluated. The clearance of $^{99m}$Tc-pentetic acid aerosols is markedly increased in both sarcoidosis and other inflammatory lung diseases. However, the limitations of $^{99m}$Tc-pentetic acids include the fact that cigarette smoking will also cause increased clearance [11]. Moreover, the pulmonary uptake of $^{99m}$Tc-HMPAO (hexamethylpropyleneaminoxoin) induced by smoking appears to be partially reversible after the cessation of smoking [12]. We evaluated the biodistribution as an experimental model to understand the action of various chemical agents [13-16]. This study deqals with possible changes in Na$^{99m}$TcO$_4$ biodistribution induced by the effects of CS in an animal model.

**Methods**

Adult male BALB/c mice were housed, 5 per cage, in a controlled environment room, with light/dark cycle conditions (12 h light/12 h dark, lights on at 6 p.m.), for an acclimatization period of $\geq$3 weeks at an ambient temperature was kept at 25 ± 2°C. The animals had free access to water and food.

Male BALB/c mice ($n = 15$), weighting 20–22 g, were exposed to smoke-air mixture of commercial filtered Virginia cigarettes, 3 times/day for 1 (CS1d), 5 (CS5d) or 10 (CS10d) days by whole body exposure in an inhalation chamber. A control group (n = 5), sham-smoke (exposed to environment air), was also used. This protocol was performed twice with a total of 40 animals, which were weighed at the end of the experiment.

Each group of mice was placed in the inhalation chamber (40 cm long, 30 cm wide and 25 cm high), inside an exhaustion chapel. A cigarette was coupled to a plastic 60 ml syringe so that puffs could be drawn in and subsequently expelled into the exposure chamber. We aspirated one litre of smoke from one cigarette with this syringe (20 puffs of 50 ml) and immediately injected the puff into the chamber. The 5 animals of each group were maintained in this smoke-air condition for 6 min (~3%), and the inhalation chamber was opened, by removing its cover, and the smoke evacuated for 1 min by exhaustion of the chapel. This cigarette exposition was repeated three times (3 × 6 min) with intervals of 1 min (exhaustion). We repeated this procedure 3 times per day (morning, lunch time and afternoon) resulting in a 54 min of CS exposition of 9 cigarettes. This protocol has been described by us elsewhere [17] and was an experimental protocol approved by the Instituto de Biologia Roberto Alcantara Gomes Animal Research Ethics Committee – UERJ.

Twenty-four hours after exposure, the mice received 0.3 ml of Na$^{99m}$TcO$_4$ (7.4 MBq) via the ocular plexus, and 30 min later, they were rapidly killed. Heart perfusion was performed with saline at constant pressure of 25 cm H$_2$O to clear organs of blood for 5 min. The organs were isolated (bones, brain, heart, intestine, kidney, liver, lungs, muscle, pancreas, spleen, stomach, testis and thyroid), their weights determined, and the organs and blood sample radioactivity of Na$^{99m}$TcO$_4$ measured by a gamma-counter NaI (TI) (Cobra Auto-gamma, Packard Instrument Co.; Downers Grove, Illinois, USA). The percentage per gram of tissue of injected dose (%ID/g) was determined for each organ. Statistical analyses involved one-way ANOVA, followed by the Tukey-Kramer Multiple Comparisons Test, with the significance level being $p < 0.05$. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego Ca, USA).

**Results**

The effects of CS on the biodistribution of Na$^{99m}$TcO$_4$ in isolated organs can first be seen in Table 1, which shows that CS decreased the %ID/g in red blood cells, bone,
kidney, lung, spleen, stomach, testis and thyroid of the exposed mice. The greatest decrease was at CS10d in kidney (p < 0.001), spleen and testis (p < 0.01). In the stomach, CS exposure changes the %ID/g for CS1d, CS5d and CS10d (p < 0.05) were comparable with the controls. Table 2 shows that CS did not change the %ID/g of Na99mTcO4 significantly in brain, heart, intestine, liver, muscle and pancreas of exposed animals.

**Discussion**

Cigarette smoke is a complex mixture of chemicals containing more than 4000 different constituents. Some of the compounds identified include pyridine alkaloids, such as nicotine, as well as ammonia, acrolein, phenols, acetaldehyde, N-nitrosamine; aromatic hydrocarbons (such as benzopyrene), combustion gases (e.g. carbon monoxide, nitrogen oxides, and hydrogen cyanide), trace metals, α-emitter radioactive elements such as polonium, radium, and thorium. Most of these compounds are produced by pyrolysis and distillation in the zone immediately behind the lighted tip of a cigarette, where the temperature reaches 950°C [18,19]. It has been estimated that undiluted mainstream smoke contains as much as 30 mg of tar and 5 mg of nicotine in an unfiltered regular tar cigarette or 0.5 mg tar and 0.05 mg nicotine in a filtered low-tar cigarette [20].

Radionuclides have been used to investigate diseases related to smoking [21,22]. In a recent study, Iwado et al. [23] demonstrated a decrease in the vasomotor response by PET (positron emission tomography) in the endothelium, in smokers. Moreover, acute CS can diminish cerebral blood flow, as was shown with technetium-99m labelled ethylcysteine in single-photon emission tomography (SPET) on 10 healthy human volunteers [24]. However, there were no changes in biodistribution in the brain in our study. However, the blood-brain barrier prevents Na99mTcO4 from entering normal brain, and the

---

**Table 1:** Organs in which CS significantly changed (%ID/g) Na99mTcO4 biodistribution (mean ± SD).

| Organs    | Groups   | Control Mean ± SD | CS1d Mean ± SD | CS5d Mean ± SD | CS10d Mean ± SD |
|-----------|----------|-------------------|----------------|----------------|-----------------|
| Red blood cells | CS10d    | 3.63 ± 0.24       | 2.73 ± 0.14*   | 2.33 ± 0.23**  | 2.74 ± 0.26*    |
| Bone      | CS10d    | 0.26 ± 0.07       | 0.24 ± 0.05    | 0.15 ± 0.07    | 0.13 ± 0.05*    |
| Kidney    | CS10d    | 0.89 ± 0.15       | 0.80 ± 0.10    | 0.62 ± 0.09*** | 0.51 ± 0.05***  |
| Lung      | CS10d    | 1.72 ± 0.68       | 1.33 ± 0.28    | 1.28 ± 0.12    | 0.86 ± 0.04*    |
| Spleen    | CS10d    | 0.36 ± 0.12       | 0.34 ± 0.06    | 0.22 ± 0.05    | 0.17 ± 0.05*    |
| Stomach   | CS10d    | 5.66 ± 1.49       | 3.53 ± 0.59**  | 3.24 ± 1.24*   | 3.37 ± 0.77*    |
| Testis    | CS10d    | 0.25 ± 0.06       | 0.19 ± 0.07    | 0.16 ± 0.03    | 0.12 ± 0.01***  |
| Thyroid   | CS10d    | 1.07 ± 0.88       | 0.80 ± 0.47    | 0.47 ± 0.38    | 0.28 ± 0.23*    |

Statistical significance: (*) p < 0.05, (**) p < 0.01, (***) p < 0.001 when compared with control.

**Table 2:** Organs in which CS unchanged (%ID/g) Na99mTcO4 biodistribution (mean ± SD).

| Organs    | Groups   | Control Mean ± SD | CS1d Mean ± SD | CS5d Mean ± SD | CS10d Mean ± SD |
|-----------|----------|-------------------|----------------|----------------|-----------------|
| Brain     | CS10d    | 0.11 ± 0.02       | 0.14 ± 0.04    | 0.11 ± 0.01    | 0.10 ± 0.03     |
| Heart     | CS10d    | 0.53 ± 0.24       | 0.61 ± 0.17    | 0.35 ± 0.12    | 0.48 ± 0.18     |
| Intestine | CS10d    | 0.91 ± 0.52       | 0.47 ± 0.21    | 0.72 ± 0.18    | 0.55 ± 0.39     |
| Liver     | CS10d    | 2.32 ± 1.30       | 1.60 ± 0.21    | 2.07 ± 0.89    | 1.20 ± 0.37     |
| Muscle    | CS10d    | 0.40 ± 0.35       | 0.19 ± 0.06    | 0.24 ± 0.21    | 0.09 ± 0.03     |
| Pancreas  | CS10d    | 0.33 ± 0.28       | 0.16 ± 0.07    | 0.11 ± 0.03    | 0.13 ± 0.05     |
radioactivity detected may represent a minimal amount of Na\textsuperscript{99m}TcO\textsubscript{4} from blood in brain circulation not cleared with our perfusion protocol.

Our experimental model showed that CS could affect %ID/g of Na\textsuperscript{99m}TcO\textsubscript{4} distribution in certain organs but not others. These changes vary according to the time of exposure; CS\textsubscript{10d} organs had lower %ID/g when compared to CS\textsubscript{5d} and CS\textsubscript{1d}, except for red blood cells, where there was lower %ID/g in CS\textsubscript{5d}. In this study, CS affected Na\textsuperscript{99m}TcO\textsubscript{4} %ID/g in red blood cells, bone, kidney, lung, spleen, stomach, testis and thyroid. It is noteworthy that some of these organs have also been associated with CS-induced cancer in humans [25].

Cigarette smoke can interfere with the ability of bone cells to participate in repairing and remodelling events [26]. This could be one of the mechanisms leading to the development of osteoporosis. Pathological analysis of the kidney by Cigremes et al. [27] has shown severe degeneration of this tissue with advanced hydrophobic degeneration of kidney tubules in the CS exposed animals.

Kidney radioactivity decreased with CS exposure as a function of time. There are two different effects of CS; first, less availability of Na\textsuperscript{99m}TcO\textsubscript{4} due to diminished blood flow to the kidney; and the second is increased permeability or secretion of Na\textsuperscript{99m}TcO\textsubscript{4}. Further studies to determine the levels of Na\textsuperscript{99m}TcO\textsubscript{4} in the urine following CS exposure may conclude whether the decrease in radioactivity is as a result of one of these above changes.

Significant dependence of alveolar deposition on flow rate, but not lung function, was found in young nonsmokers compared with CS\textsubscript{1d}. The CS\textsubscript{1d} organs had lower %ID/g when compared to CS\textsubscript{5d} and CS\textsubscript{1d}, except for red blood cells, where there was lower %ID/g in CS\textsubscript{5d}. In this study, CS affected Na\textsuperscript{99m}TcO\textsubscript{4} %ID/g in red blood cells, bone, kidney, lung, spleen, stomach, testis and thyroid. It is noteworthy that some of these organs have also been associated with CS-induced cancer in humans [25].

When comparing the sensitivity of CS in BALB/c mice to Na\textsuperscript{99m}TcO\textsubscript{4} biodistribution, we cannot exclude the possibility that C57BL6 mice will give better results in the future. When Na\textsuperscript{99m}TcO\textsubscript{4} is used as a powerful diagnostic tool in nuclear medicine for examining patients to assess the brain and thyroid, CS can cause alterations in the readings.

**Conclusion**

CS induced changes of Na\textsuperscript{99m}TcO\textsubscript{4} biodistribution in exposed BALB/c mice. Na\textsuperscript{99m}TcO\textsubscript{4} distribution on kidney, lung, spleen, testis and thyroid decreased progressively with CS time exposure from 1 to 10 days. The Na\textsuperscript{99m}TcO\textsubscript{4} distribution in stomach and red blood cells was affected from the first day of CS exposure.

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors’ contributions**

SSV – carried out the experimental procedure with the mouse exposed to CS, performed the statistical analysis and write the manuscript.

Rajpurkar et al. [31] analysed chronic cigarette smoke on rat testis, and observed apoptosis, which may be one of the pathogenic mechanisms responsible for defective spermatogenesis in these animals, Fisher et al. [32] showed that chronic smokers have higher thyroxin levels and a lower thyroid-stimulating hormone level than non-smokers or former smokers. These results corroborate the effects on specific organs by CS, which validates this experimental design as a method to seek alterations of Na\textsuperscript{99m}TcO\textsubscript{4} biodistribution in mice.
EACL – carried out the experimental procedure with Na$^{99m}$TcO$_4$ (7.4 MBq) via the ocular plexus.

GFD – determined the radioactivity of the Na$^{99m}$TcO$_4$ in a counter NaI (TI)

MB-F – conceived of the study and participated in its design and coordination.

LCP – conceived of the study and participated in its design and coordination.

All authors read and approved the final manuscript.

Acknowledgements
This research was supported by Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References
1. Rahman I, MacNee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease. Thorax 1996, 51:348-350.
2. Peto R, Lopez AD, Goreham J, Thun M, Heath CJr: Mortality from tobacco in developing countries: indirect estimation from national vital statistics. Lancet 1992, 339:1268-1278.
3. World Health Organization Mortality Statistics, World Health Statistics Annual 1997–1999 [http://www.who.int/whosis/mort]
4. Hom RK, Karzenellenbogen JA: Technetium-99m labeled Receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results. Nucl Med Biol 1997, 24:485-498.
5. Hladik J, Snozzi M, Bachofen R: Incorporation of reaction centers into sub mitochondrial particles resulting in light induced electron transfer. Biochem Biophys Res Commun 1987, 14:170-177.
6. Early P, Sodee DB: Principles and practice of nuclear medicine Moosby-Year Book; London: 1995.
7. Saha GB, Go RT, MacIntyre WJ: Radiopharmaceuticals for cardiovascular imaging. Int J Radiat Appl Instrum B 1993, 19:1-20.
8. Hesselwood S, Leung E: Drug interactions with radiopharmaceuticals. Eur J Nucl Med 1994, 21:348-356.
9. Sampson CB: Adverse reactions and drug interactions with radiopharmaceuticals. Drug Safety 1993, 8:280-294.
10. Lentle BC, Scott JR: Iatrogenic alterations in radionuclide biodistribution. Semin Nucl Med 1979, 9:131-143.
11. Baughman RP, Fernandez M: Radionuclide imaging in interstitial lung disease. Curr Opin Pulm Med 1996, 2:376-379.
12. Shih WJ, Lee JK, Coupal J, Lai YL, Gruenwald F, Biersack H: Diminished Tc-99m HMPAO pulmonary uptake in ex-smokers. Clin Nucl Med 1995, 20:788-791.
13. Capriles PVSS, Dias AP, Costa TEMH, Oliveira MBN, Faria MVC, Moura EG, Abreu BAL, Bernardo-Filho M: Effect of eggplant (Solanum melongena) extract on the in vitro labeling of blood elements with technetium-99m and on the biodistribution of sodium pertechnetate in rats. Cell Mol Biol (Noisy-le-grand) 2002, 48:771-776.
14. Feliciano GD, Lima EAC, Pereira MJDS, Oliveira MBS, Moreno SRF, Mattos DMH, Jales RL, Bernardo-Filho M: Effect of a chayotte (sechium edule) extract on the labeling of red blood cells and plasma proteins with technetium-99m: in vitro and in vivo studies. Cell Mol Biol (Noisy-le-grand) 2002, 48:751-755.
15. Gomes ML, Braga ACS, Mattos DMH, Freitas RS, Boassiquevisque E, Bernardo-Filho M: The effect of mitomycin-C on the biodistribution of $^{99m}$Tc-MDP in Balb/c mice. Nucl Med Commun 1998, 19:1177-1183.
16. Gomes ML, Mattos DM, Freitas RS, Dire GF, Lima EA, Souza SM, Bernardo-Filho M: Evaluation of the effect of mitomycin-C on the bioavailability of technetium-99m-labelled sodium pyrophosphate in mice. Cell Mol Biol (Noisy-le-grand) 2002, 48:757-759.
17. Valenga SS, da Hora K, Castro P, Moraes VG, Carvalho L, Porto LC: Erythrosine and methylolacetate expression in lung induced by cigarette smoke. Toxicol Pathol 2004, 32:351-356.
18. Rodgman A, Smith CJ, Perfetti TA: The composition of cigarette smoke: a retrospective, with emphasis on polycyclic components. Hum Exp Toxicol 2000, 19:573-595.
19. Skwarszcz B, Ulatowski J, Struminska DI, Borylo A: Inhalation of 210Po and 210Pb from cigarette smoking in Poland. J Environ Radioact 2001, 57:221-230.
20. Hoffmann D, Hoffmann I: The changing cigarette, 1950–1995. J Toxicol Environ Health 1997, 50:367-364.
21. Meeder JG, Blanksm PK, van der Wall EE, Anthonio RL, Willemsen AT, Prum J, Vaalburg W, Lie KL: Long-term cigarette smoking is associated with increased myocardial perfusion heterogeneity assessed by positron emission tomography. Eur J Nucl Med Mol Imaging 2002, 29:984-990.
22. Yamamoto Y, Nishiyama M, Monden T, Satoh K, Oikawa M: A study of the acute effect of smoking on cerebral blood flow using $^{99m}$Tc-ET-ECP SPET. Eur J Nucl Med Imaging 2003, 30:612-614.
23. De Flora S, D’Agostini F, Balansky R, Camoirano A, Bennicelli C, Bagnasco M, Cartiglia C, Tampo E, Longobardi MG, Luber RA, Izziotti A: Modulation of cigarette smoke-related end-points in mutagenesis and carcinogenesis. Mutat Res 2003, 523-524:237-252.
24. Liu X, Kohyama T, Kobayashi T, Abe S, Kim HJ, Reed EC, Rennard SI: Cigarette smoke extract inhibits chemotaxis and collagen gel contraction mediated by human bone marrow osteoprogenitor cells and osteoblast-like cells. Osteoporsis Int 2003, 14:235-242.
25. Cigremis Y, Turkoz Y, Akgoz M, Sozmen M: The effects of chronic exposure to ethanol and cigarette smoke on the level of reduced glutathione and malondialdehyde in rat kidney. Urol Res 2004, 32:213-218.
26. Agnew JE, Pavia D, Clarke SW: Factors affecting the alveolar deposition of 5 microns inhaled particles in healthy subjects. Clin Phys Phisiof Meas 1985, 6:273-6.
27. Sopori ML, Gairola CC, DeLucia AJ, Bryant LR, Cherian S: Immune responsiveness of monkeys exposed chronically to cigarette smoke. Clin Immunol Immunopathol 1985, 36:338-344.
28. Shin YY, Wang HY, Liu ES, Koo MW, Cho CH: Differential effects of cigarette smoke extracts on cell proliferation in gastric and colon cells. Cancer Invest 2003, 21:200-207.
29. Ralpurkar A, Jiang Y, Dhabuwala CB, Dunbar JC, Li H: Cigarette smoking induces apoptosis in rat testis. J Environ Pathol Toxicol Oncol 2002, 21:243-248.
30. Fisher CL, Mannino DM, Herman WH, Frumkin H: Cigarette smoking and thyroid hormone levels in males. Int J Epidemiol 1997, 26:972-977.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2385/5/1/prepub