International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Research Article **Open Access**

Incidental Renal Findings during ^{99m}Tc-MDP Biological Distribution Assay. The Role of Small Animal Imaging.

Portillo, Mariano Gastón*, Tesán, Fiorella Carla, Giaquinta Romero, Diego, Zubillaga, Marcela Beatriz, Salgueiro, María Jimena

Radioisotope Laboratory, Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina.

A B S T R A C T

The present work aims to report an incidental renal finding during a bone scintigraphy performed as an additional exploratory study during a biological distribution assay as part of the quality control of 99mTc-labeled methylene disphosphonate (99mTc-MDP). Radiolabelling and quality control of 99mTc-MDP were performed according to the instructions of the manufacturer as well as to the USP monograph. After euthanasia and prior to dissection in the biological distribution, bone scintigraphy was carried on using a small field of view gamma camera. Finally, after dissection images of the kidneys were acquired. The uptake of 99mTc-MDP in the kidneys was calculated as the uptake of each kidney with regard to total renal uptake. Imaging analysis showed a remarkable difference in 99mTc-MDP uptake between right and left kidney (15.0% and 85.0% respectively). Lateral views confirmed anatomical positioning of kidneys and counting. Confirmation of the differential uptake in kidneys of the radiopharmaceutical was also achieved by *ex vivo* techniques [92.8% for left kidney and 7.2% for right kidney measured *ex vivo* in gamma camera; and 91.6% for left kidney and 8.4% for right kidney measured in a well-type gamma counter]. The present work clearly demonstrates that the comprehensive assessment of the biological behavior of radiopharmaceuticals assayed in experimental animals by using the small animal imaging approach, contributes more efficiently to the interpretation of the results. In this case, it was possible to detect incidental renal abnormalities that influenced the outcome of the biological distribution assay of 99mTc-MDP.

Keywords: 3Rs, 99mTc-MDP, biological distribution, radiopharmaceuticals, renal findings, small animal imaging.

A R T I C L E I N F O

CONTENTS

Article History: Received 15 September 2014, **Accepted** 10 November 2014, **Available Online** 10 February 2015

***Corresponding Author**
Portillo, Mariano Gastón
Radioisotope Laboratory, Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina.
Manuscript ID: IJMPR2330

PAPER-QR CODE

Citation: *Portillo, Mariano Gastón, et al.* Incidental Renal Findings during 99mTc-MDP Biological Distribution Assay. The Role of Small Animal Imaging.. *Int. J. Med. Pharm, Res.*, 2015, 3(1): 891-896.

1. Introduction

Since radiopharmaceuticals are generally prepared for intravenous human administration, quality control of the product is mandatory as in the case of any drug for injection. Moreover quality control of radiopharmaceuticals is carried out in the context of a program of quality assurance that radiopharmacies accomplish for their operation [1, 2]. Basically, quality control involves several tests that are applied to nonradioactive pharmaceuticals in addition to tests that are specific for radiopharmaceuticals [3, 4].

These tests are grouped in two categories: physicochemical such as radionuclidic and radiochemical purities, pH, ionic strength, osmolality, and physical state of the sample, and biological tests such as sterility, apyrogenicity, bacterial endotoxins, biological distribution and toxicity [3, 4]. In this way, purity, potency, product identity, biologic safety, and efficacy are guaranteed. Routinely, quality control tests are performed by the manufacturers at the industrialized radiopharmacies [5-8]. However, the introduction of cold kits for radiolabelling with short-lived radionuclides such as 99mTc, as well as the on-site compounded of many radiopharmaceuticals at the hospital radiopharmacy require that most, if not all, quality control tests be performed on all preparations before dispensing these products for human administration [5-9].

In particular, biological distribution assay is one of the most important parts of the development and routine testing processes of many radiopharmaceuticals, like 99mTc-Medronate (99mTc-MDP). The objective of the test is to verify the biodistribution and metabolic properties of a radiopharmaceutical, when the exact chemical structure of the complex is not fully known [10]. The individual monograph, usually encoded in the pharmacopoeia, prescribes the details concerning the technical recipe of the assay and the physiological distribution requirements, which must be met for the radiopharmaceutical preparation [11-13]. Briefly, this kind of assays are performed on 3 mice or rats that are administered with the radiopharmaceutical intravenously and at the end time point, the uptake in specific organs is measured and results are compared to the established limits to declare if the radiopharmaceutical complies the requirements, so it is acceptable for its use [14]. Usually limits must be accomplished in at least 2 of the three animals involved [14]. It is noteworthy that biological distribution assays require the sacrifice of the animals, except in a few cases, because the organs of interest are dissected in order to be measured in a dose calibrator or in a well-type gamma counter.

In this sense, since accomplishment of health directives requires the performance of assays that employ experimental animals, it is possible that in the near future they may benefit from the emergence and explosive expansion of small animal imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) [15, 16]. The use of these techniques will not only contribute to the welfare of the animals according to the 3R concept [replacement-reduction -refinement] but also will provide the possibility of more comprehensive studies on the biodistribution and metabolism of radiopharmaceuticals [17-19].

In addition, the possibility to explore the physiology of the animal used as a biological reagent, will allow the validation of the results obtained in the in vivo context that is also more detailed and inclusive than that of the ex vivo [17-19]. This is especially remarkable given the particular characteristic of the radiopharmaceuticals, which are classified as true functional probes. The present work aims to report an incidental renal finding during a bone scintigraphy performed as an additional exploratory study during the routine of a biological distribution assay as part of the quality control of 99mTc-MDP. The contribution of the results to the topic introduced above is also discussed.

2. Materials and Methods

Animal procedures were in accordance with international recommendations (Guide for the Care and Use of Laboratory Animals of the Research Council, USA, 1996; Guidelines for the welfare and use of animals in cancer research British Journal of Cancer, 2010) and protocols were approved by Ethical Committee for the Use and Care of Laboratory Animals of the School of Pharmacy and Biochemistry (EXP-FYB N° 53406/2013). Radiolabelling of 99mTc-MDP: it was performed by the addition of a sterile solution of sodium pertechnetate recently eluted from a $^{99}Mo/^{99m}Tc$ generator (Laboratories BACON SAIC, Argentina) to a cold kit (BONE TEC ®, Tecnonuclear SA, Argentina), according to the instructions of the manufacturer. Sodium pertechnetate quality control was performed previously to the radiolabelling, according to the USP monograph [20]. Physicochemical quality controls of 99mTc-MDP: the appearance was assayed according to the instructions of the manufacturer and the other physicochemical tests such as determination of pH and radiochemical purity were performed according to the USP monograph [12]. Biological distribution assay of 99mTc-MDP: it was accomplished according to the USP monograph of 99mTc-MDP [12].After euthanasia and prior to dissection, bone scintigraphy was carried on as described

below. Additionally, radioactivity of each kidney was measured in the calibrated well-type gamma counter and results are expressed as: % Uptake kidney$_{[right\ or\ left]}$= Uptake kidney$_{[right\ or\ left]}$ x 100/ Uptake right kidney + Uptake left kidney, 99mTc-MDP bone scintigraphy: a small field of view gamma camera (OhioNuclear, Model Sigma 420, USA) with dedicated software (IM512P v.3.3, Alfanuclear SAIC, Argentina) associated was used. Static images were acquired using a high resolution parallel hole collimator in a matrix of 256 x 256 and zoom x 1.25 with the window centered on the peak of the 99mTc with a width of 15-20%. Acquisitions were performed in order to achieve at least 10^6 counts. Animals were positioned in ventral decubitus and lateral incidences were also acquired. The images were visually analyzed and regions of interest (ROIs) were created on the ventral image projection of the renal silhouettes. Finally, after dissection images of the kidneys were acquired in the same conditions that the whole body incidence just changing the zoom x 2 during 5 minutes and also ROIs were created for each kidney.The uptake of 99mTc-MDP in the kidneys was calculated as described above using data of ROIs.

3. Results and Discussion

Normal biological distribution pattern over the bones, the urinary tract and the accumulated urine in the bladder of 99mTc-MDP 1 hour post-injection is shown in Figure 1. Incidental renal findings are shown in Figure 2, where there is a remarkable difference in 99mTc-MDP uptake between right and left kidney. Lateral views were acquired in order to confirm anatomical positioning of kidneys. They showed bone and soft-tissue uptake of 99mTc-MDP in abdomen as well as in left kidney uptake almost over the vertebrae column while right kidney uptake was almost negligible (data not shown).

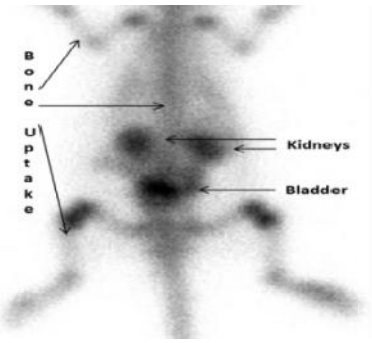

Figure 1: Normal biological distribution pattern of 99mTc-MDP 1 hour post-injection.

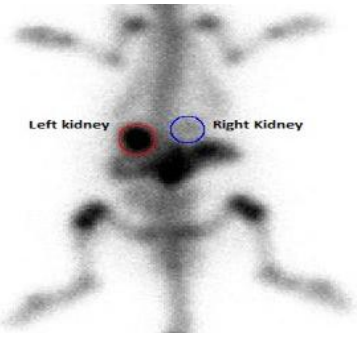

Figure 2: Incidental renal findings. Remarkable difference in 99mTc-MDP uptake between right and left kidney

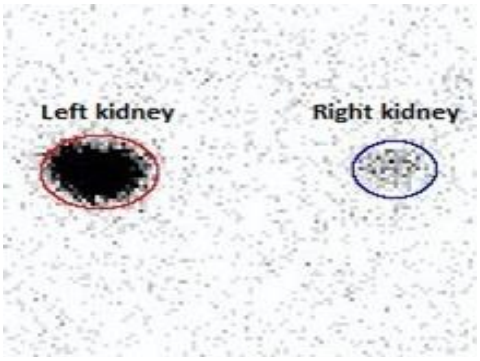

Figure 3: Uptake and distribution of 99mTc-MDP in both kidneys [ex vivo].

When both kidneys were dissected and their image was acquired, confirmation of the differential uptake of the radiopharmaceutical was achieved showing that the counting and the size of both organs is clearly different (figure 3). Table 1 summarizes uptake values obtained by different semiquantitative methods. Kidney uptake measured from the ROIs drawn on the images acquired in gamma camera were 85% for left kidney and 15% for right kidney; when it was measured ex vivo in gamma camera left kidney uptake was 92.8% while right kidney showed 7.2% and when uptake was measured ex vivo in a well-type gamma counter it resulted 91.6% for left kidney and 8.4% for right kidney.

Table 1: Renal uptake values by semiquantitative methods *in vivo* and *ex vivo*. a) kidney uptake measured from the ROI drawn on the images acquired in gamma camera b) kidney uptake measured *ex vivo* in gamma camera c) kidney uptake measured *ex vivo* in a well-type gamma counter.

	Rois of *in vivo* image %	**Rois of *ex vivo* image %**	***Ex vivo* counting %**
Left Kidney	85.0	92.8	91.6
Right Kidney	15.0	7.2	8.4

Discussion:

The 99mTc-MDP (metilen diphosphonate or medronate) is a radiopharmaceutical customarily used in the clinic for the evaluation of bone disease such as: a) bone secondary metastases to a wide variety of tumors, b) infections, c) fractures, etc [21]. The mechanism of localization of the

complex 99mTc-MDP in bone is not completely known but it is widely accepted that it fixes on bone surface by quimioadsorption [21]. After intravenous administration, 99mTc-MDP uptake depends on blood flow and removal from the vascular space to the bone. Blood clearance is fast, and approximately 50% of the administered dose is eliminated in urine during the first 24 hours without degradation of the 99mTc-MDP complex [22].

This is in direct correlation with clearance needed for image quality. In Argentina, the quality control of 99mTc-MDP is carried out within the framework of a quality assurance program in industrialized radiopharmacies. Thus, safe application of radiopharmaceuticals is in accordance with normative both in the field of radiation protection and sanitary standards [23-26]. In this context biological distribution assay is performed prior to the marketing of each batch of cold kits for preparing 99mTc-MDP. In this work, two important issues related to the use of animals as biological reagents arise from the results obtained. The first one regards to the quality of the animals involved in the assay and how this may interfere with the results, and ultimately, with the quality of the radiopharmaceutical that is going to be commercialized. The second issue, and intimately related to the first, is derived from the 3Rs concept and suggests thatthe technical refinement from euthanasia and dissection to the image would be not only useful for the strengthening of the concept but also may add clear benefits at the time of analyzing the results.

The present work clearly demonstrates that the comprehensive assessment of the biological behavior of the radiopharmaceutical assayed in experimental animals by using the small animal imaging approach, contributes more efficiently to the interpretation of the results. In this case, it was possible to detect incidental renal abnormalities that influence the outcome of the assay. Renal findings are usually reported while performing bone scintigraphy in humans [27, 28].This is because the renal elimination of 99mTc-MDP. Imagenological patterns of renal findings are extensively described in literature and this information contributes to the comprehensive evaluation of the patient by the physician, so that they became an integral part of the nuclear medicine report [27, 28]. In this case, renal imagenological findings belong to the category of no visualization of one kidney which may be related with a chronic progressive nephropathy (CPN) condition. CPN is a renal disease affecting all conventional strains of laboratory rats [29, 30], for example Sprague Dawley strain commonly used in Argentina and also indicated as the strain required for many biological distribution assays. Features of CPN are well reviewed in literature [29] and it is noteworthy that diagnose is not simply revealed by common laboratory tests, especially during early-stage disease, since blood urea nitrogen and creatinine are not affected and urinary proteins may not be related to CPN but to other conditions according to contemporary debate about CPN pathogenesis. Additionally, although in our country there is specific normative [31, 32] to assure the quality of animal production, efforts are mainly directed to assay bacterial, viral, parasitic and zoonotic diseases. A discussion that might be done is that for some special uses of laboratory animals, it would be desirable to advance another step in the diagnosis or the detection of the abnormalities that could invalidated the individual as a health biological reagent. In this sense, small animal imaging techniques are one of the options.

Thus, the introduction of small animal imaging launches an scenario in which according to the USP biological distribution assay, the uptake of 99mTc-MDP by both kidneys complies with the specified requirement of < 5%, as obtained by the *ex vivo* radioactivity measurement. Nevertheless, the contribution of the image tells us that one of the kidneys is functionally affected. In fact, CPN results in hyperfiltration which [29, 30] is compatible with the no visualization of the kidney but also may be responsible of the lower kidneys uptake (< 5%) required in USP monograph [12]. In this way, the certainty of the result of quality control is controversial because of the imaging findings. Therefore, is this assay technique, as it is stated using euthanasia and dissection, sufficiently specific and sensitive to provide information about the characteristics of the tested radiopharmaceutical quality. It is worthwhile to explore imaging contribution to this kind of assays not only in terms of biological information provided but also in the context of welfare of laboratory animals and 3Rs concept.

In our experience, the acquisition of images during the execution of the biological distribution assay allows a quick review of the imagenological pattern of biodistribution of the radiopharmaceutical in the animal, providing closely-related information to that which is obtained in patients at the Nuclear Medicine Service. In addition, it allows the validation of the results obtained during quality control since it permits the comprehensive assessment of the biological reagent (rat) involved in the trial. On the other hand, this opens the future perspectives of possible replacement of the former dissection techniques for imaging techniques that provide not only integral information about the biological behavior of the radiopharmaceutical but also contribute to the development of the concept of the 3Rs in the work with experimental animals [33, 34].

4. Conclusion

The possibility of comprehensive analysis of individuals involved as well as identification of true outliers during the course of biodistribution testing protocols for radiopharmaceuticals through imaging, supports the complementarily of diagnostic images and *ex vivo* techniques, for the validation of data in both directions. The migration to imaging techniques when working with laboratory animals is an interesting possibility to explore nowadays, for strengthening both research results and animal welfare. Biological distribution assays for radiopharmaceuticals quality control may extensively benefit from imaging techniques.

5. Acknowledgements

This work was financially supported by project UBACYT 20020100100489 from the University of Buenos Aires.

6. References

1. Beaney A. In: *Sampson´s textbook of radiopharmacy*; Theobald T ed.; Pharmaceutical press: London, UK, **2011:** pp. 365-370.
2. Bringhammar T, Zolle I. In: *Technetium-99m Pharmaceuticals*; Zolle I. ed; Springer: Berlin Heidelberg, **2007**, pp 67-71.
3. Maltby P, Theobald T. In: *Sampson´s textbook of radiopharmacy*; Theobald T. ed.; Pharmaceutical press: London, UK, **2011,** pp. 371-420.
4. Saha G. In: *Fundamentals of nuclear pharmacy*; Saha G ed.; Springer: New York, USA, **2010,** pp. 153-74.
5. Decristoforo C. In: *Sampson´s textbook of radiopharmacy*; Theobald T. ed.; Pharmaceutical press: London, UK, **2011,** pp. 483-494.
6. Hartman NG. In: *Sampson´s textbook of radiopharmacy*; Theobald T. ed.; Pharmaceutical press: London, UK, **2011,** pp. 495-500.
7. Hung JC. In: *Sampson´s textbook of radiopharmacy*; Theobald T. ed.; Pharmaceutical press: London, UK, **2011,** pp. 501-524.
8. Saha G. In: *Fundamentals of nuclear pharmacy*; Saha G ed.; Springer: New York, USA, **2010**; pp. 175-92.
9. Saha G. In: *Fundamentals of nuclear pharmacy*; Saha G ed.; Springer: New York, USA, **2010**, pp. 207-38.
10. Preparacionesradiofarmacéuticas1100. FA VIII edición, III volumen http://www.anmat.gov.ar/webanmat/fna/octava_edicion/Tercer_Volumen.pdf [Accessed May 16, **2014**, pp. 586.
11. Tecnecio [99mTc] Medronato de Solución inyectable. FA VIII edición, III volumen .http://www.anmat.gov.ar/webanmat/fna/octava_edicion/Tercer_Volumen.pdf[Accessed May 16, **2014**, pp. 623.
12. Technetium Tc 99m Medronate Injection. USP (The United States Pharmacopeial Convention) 36 NF (U.S. Pharmacopeia National Formulary) 31, segundosuplemento, **2013,** pp.5292 .
13. Technetium (99mTc) medronate injection. EP (European Pharmacopoeia) 8.0. **2014**, pp 1102-3.
14. Technetium-99m radiopharmaceuticals: manufacture of kits. International Atomic Energy Agency, Vienna, Austria, 2008. Technical Reports Series no 466. Annex X: Biodistribution Studies, pp186-187.
15. Southworth R. In: *Sampson´s textbook of radiopharmacy*; Theobald T. ed.; Pharmaceutical press: London, UK, **2011,** pp. 617-632.
16. Zanzonico P. In: Small *animal imaging. Basics and practical guide*; Kiessling F, Pichler B. Eds, Hauff P Co-ed.; Springer: Berlin, Germany, **2011**; pp 3-16.
17. Ricketts SA, Hockings PD, Waterton JC. In: Small *animal imaging. Basics and practical guide*; Kiessling F, Pichler B. Eds, Hauff P Co-ed.; Springer: Berlin, Germany, **2011**; pp 17-28.
18. Beckmann N, Maier P. In: Small *animal imaging. Basics and practical guide*; Kiessling F, Pichler B. Eds, Hauff P Co-ed.; Springer: Berlin, Germany, **2011**; pp 47-57.
19. Ulbrich HF. In: Small *animal imaging. Basics and practical guide*; Kiessling F, Pichler B. Eds, Hauff P Co-ed.; Springer: Berlin, Germany, **2011**; pp 69-82.
20. Technetium Tc 99m Medronate Injection. USP (The United States Pharmacopeial Convention) 36 NF (U.S. Pharmacopeia National Formulary) 31, Segundo suplemento, **2013,** pp.5296
21. Kowalsky, R. Falen, S. In: *Radiopharmaceuticals in nuclear pharmacy and nuclear medicine*. APhA: Washington DC, **2004**, pp. 671-91.
22. Jovanocic, V. Maksin, T. Rastovac, M. Bzenic, J. Comparative quality control of 99mTc-Pyrophospate and 99mTc-Diphosphonate Radiophamaceuticals.*Eur J Nucl Med*, ***1983***, *8*, 179-182.
23. Disposición Nº 2819/04. B.O.: 07/06/04 Lineamientos Generales de Buenas Prácticas de Fabricación para Elaboradores, Importadores/ Exportadores de Medicamentos.
24. Disposición N° 2009/07. B.O.: 13/04/07Pautas, documentación y requisitos a presentar para solicitar la autorización de los medicamentos destinados a ser administrados a seres humanos con fines diagnósticos o de monitoreo denominados Productos para Diagnóstico de uso "*in vivo*".
25. Norma AR 10.1.1. B.O.: 21/11/01. Norma Básica de Protección Radiológica rev 3. Autoridad Regulatoria Nuclear (ARN). http://www.arn.gov.ar/images/stories/que_hace_la_ARN/resena_de_actividades/marco_regulatorio/normas_regulatorias/10.1.1_R3.pdf. [Accessed May 26, 2014].
26. Norma AR 8.2.4. B.O.: 22/07/02. Uso de fuentes no selladas en instalaciones de Medicina Nuclear rev 1. Autoridad Regulatoria Nuclear (ARN). http://www.arn.gov.ar/images/stories/que_hace_la_ARN/resena_de_actividades/marco_regulatorio/normas_regulatorias/8.2.4_R1.pdf. [Accessed May 26, **2014**].
27. Gentili A, Stefan DM, Lee P. Incidental detection of urinary tract abnormalities with skeletal scintigraphy. *Radiographics*, ***1991,*** *11*, 571-579.
28. Loufti I, Collier BD, Mohammed AM. Nonosseus abnormalities on bone scans. *J NuclMedTechnol*, ***2003***, *31*: 149-153.

29. Hard GC, Khan KN. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *ToxicolPathol*, **2004**, 32, 171-180.
30. Hard GC, Johnson KJ, Cohen SM. A comparison of rat chronic progressive nephropathy with human renal disease. Implications for human risk assessment.*Crit Rev Toxicol*, ***2009***, *39*, 332-346.
31. Resolución (SENASA) Nº617/02. B.O.: 24/7/2002. Requisitos, condiciones y procedimientos para la habilitación técnica de laboratorios que posean bioterios de producción, mantenimiento y local de experimentación. Informe de ensayo de residuos de productos fitosanitarios en matrices vegetales. Informe de campo. Informe analítico.
32. Disposición (ANMAT) N°6344/97. B.O.: 20/01/97. Reglamentación para bioterios de laboratorios elaboradores de especialidades medicinales y/o de análisis para terceros.
33. Directive 2010/63/EU of The European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.http://eurlex.europa.eu/legalcontent/EN/TXT/?uri=CELEX:32010L0063[Accessed April 10, **2014].**
34. Guide for the care and use of laboratory animals. The National Academies Science. 2011. The National Academies Press, New York, USA. http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf**.** [Accessed March 26, **2014**].