2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-05-2**

ACUTE AND SUBACUTE TOXICITY OF ^{18}F-FDG

Danielle M. Dantas[1], Natanael G. da Silva[2], Ana Paula Manetta[3] and João A. Osso Jr.[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
danielle_2705@hotmail.com
jaossoj@yahoo.com.br

[2] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
ngsilva@ipen.br

[3] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
apaulasp2008@hotmail.com

ABSTRACT

Before initiating clinical trials of a new drug, it is necessary to perform a battery of safety tests, for evaluating the risk in humans. Radiopharmaceuticals must be tested taking into account its specificity, duration of treatment and especially the toxicity of both, the unlabelled molecule and its radionuclide, apart from impurities emanating from radiolysis. In Brazil the production of radiopharmaceuticals was not regulated until the end of 2009, when ANVISA established the Resolutions No. 63, which refers to the Good Manufacturing Practices of radiopharmaceuticals and No. 64 which seeks the registration of radiopharmaceuticals. Nowadays IPEN produces one of the most important radiopharmaceutical for nuclear medicine, the ^{18}F-FDG, which is used in the diagnosis. The objective of this study is to assess systemic toxicity (acute / subacute) of ^{18}F-FDG in an in vivo test system, as recommended by the RDC No. 64. In acute tests the administration occurred on the first day, healthy rats were observed for 14 days reporting their clinical signs and water consumption, and on the 15th day they were euthanized and necropsied. The assay of subacute toxicity observations were made over a period of 28 days and the first dose was administered at the beginning of the test and after a fortnight a second dose was administered. The parameters evaluated were the necropsy, histopathology of target organs, hematology studies and liver and kidney function. The results are being processed and evaluated. Initial observations did not show any acute toxicity in animals when compared to control animals.

1. INTRODUCTION

Radiopharmaceuticals that are administered in amounts that exceed the limits of a microdose mass or which are intended to different authorities, must fully meet the requirements of safety studies of non-clinical testing - preclinical studies in vitro and in vivo - as any active substance before being used in human clinical trials [1].

In Brazil, ANVISA has its own regulations. By the end of 2009 there was no specific regulations facing radiopharmaceuticals. A working group formed by ANVISA and the IPEN was created in late 2006, in order to develop specific regulations for the production of radiopharmaceuticals and registration [2].

At the end of 2009 the resolutions No. 63 and No. 64 granted to radiopharmaceuticals were published. The RDC 63 deals with the Good Manufacturing Practices for Radiopharmaceuticals by setting minimum requirements to be met in order to obtain a radiopharmaceutical within the quality standards [3]. The RDC No. 64 establishes the requirements for the registration process of radiopharmaceuticals in the country in order to ensure the quality, safety and efficacy of this drug [4]. The registration process should report the radiopharmaceutical preclinical acute, subacute and chronic toxicity, reproductive, mutagenic and carcinogenic potential in accordance with the law [4].

2. OBJECTIVE

The purpose of this study is to evaluate the systemic toxicity (acute/subacute) of the diagnostic radiopharmaceutical ^{18}F-FDG in an in vivo test system, as recommended by the RDC No. 64 of ANVISA.

The legislation adopted for this work will be the guides for the pharmaceutical industry ANVISA.

3. BACKGROUND

The knowledge gained in this work will be an important step in learning the toxicity testing of radiopharmaceuticals produced at IPEN, aiming at the development of protocols for toxicity. The choice of radiopharmaceutical ^{18}F-FDG reflects its importance for the Brazilian Nuclear Medicine.

Currently, ^{18}F-FDG is used in the majority of diagnoses obtained through the technique of PET, because it allows greater sensitivity in the images obtained, more accurate diagnoses and broad spectrum detection of oncological diseases, cardiac and neurological.

4. MATERIALS AND METHODS

4.1 Infrastructure and Equipment

All procedures were performed in the laboratories of the Center of Radiopharmacy (CR) and the Biotechnology Center at the Institute of Energy and Nuclear Research (IPEN-CNEN/SP). Out of IPEN outsourced services were performed in Lab & Vet.

4.2 Evaluation of the Injected Dose of ^{18}F-FDG

This study aimed to define the dose of activity to be administered in the acute toxicity test (single dose) and subacute toxicity test (multiple doses). The acute and subacute toxicity studies had the aim of evaluating the toxic effects of the pharmaceutical and its metabolites, not the radioactivity effects. So, the doses were injected in the animals after the complete decay of ^{18}F [5].

The dose administered in the animals was calculated based on the maximum dose of activity that a patient weighing 70 kg receives in an exam, about 550MBq (15 mCi), standard dose of Nuclear Medicine services in Brazil. This dose was calculated and converted to rats of 300 g and thus establishing a standard dose for cold radiopharmaceutical activity, the volume and mass of injected radiopharmaceutical.

4.3 Acute Toxicity Studies

Three groups of healthy Wistar rats were studied: Control group (3 females and 3 males), were injected with saline; 100X group (3 females and 3 males), injected with 100 times the standard dose and the 1000X group (3 females and 3 males), injected with 1000 times the standard dose of the cold radiopharmaceutical. The following parameters were observed: body weight, clinical signs and water consumption of the animals for 14 days, and on the 15th the animals were euthanized for autopsy examination and histopathology of target organs (heart, brain, liver, kidneys and bladder). The results were compared using ANOVA by Dunnett's test. Euthanasia was performed with a combination of ketamine + xylazine anesthetic doses slightly higher than the standard, and finally ending with a CO_2 chamber.

4.4 Subacute Toxicity Studies

The administration was performed in 4 groups of healthy Wistar rats: control group (3 females and 3 males), Glucose Group (3 females and 3 males), 100X Group (3 females and 3 males) and 1000X Group (3 females and 3 males). The observations were carried out for a period of 28 days. A first dose was administered at the beginning of the test and after 14 days a second dose was administered, exposing the healthy rats to two doses. The parameters evaluated in this study were: weight, water and food consumption, necropsy, histological examination of target organs (heart, brain, liver, bladder and kidneys), blood count and liver and kidney function, where all groups were compared with the group control using ANOVA by Dunnett's test.

5. RESULTS AND DISCUSSION

5.1 Evaluation of the Injected Dose of ^{18}F-FDG

A standard dose of 550MBq (15 mCi) for the diagnosis of a 70 kg adult patient was chosen. This dose corresponds 2.36 MBq (64μCi) to a mouse of 300 g. It was assumed a total of 3 administrations per year, corresponding to 7.10MBq (192 μCi) per animal. Doses of 100X and 1000X corresponded to 710 MBq (19.2 mCi) and 7.10MBq (192 μCi), respectively.
It was waited for the full decay of ^{18}F before starting the injection in animals.

5.2 Acute Toxicity Studies

5.2.1 Body Weight

Figure 1 shows the weight variation for all groups. A total of 5 weightings were made during the 15 days of experiment. The results showed that the variation in weight was observed for most of the animals after the injection in the first 3 days and that there was a small decrease in weight that does not vary more than -3%. The weight change of females and males were

not statistically significant, so no signs of toxicity from the administration of ^{18}F-FDG were found.

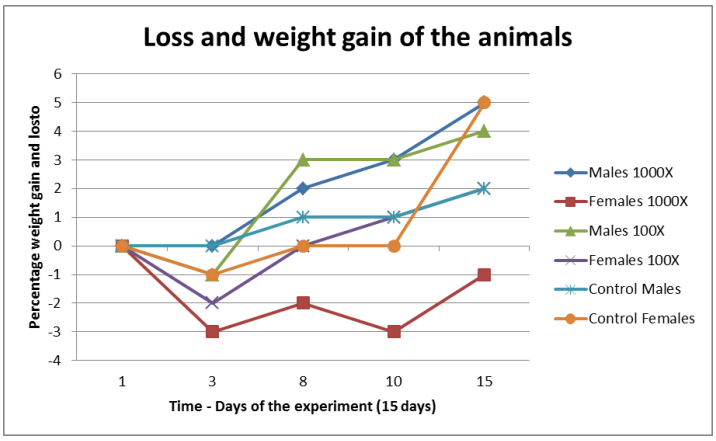

Figure 1: Weight variation for the control, glucose, 100X and 1000X groups for the acute toxicity test.

5.2.2 Water consumption

The water consumption was observed per box, and the results are shown in figure 2.

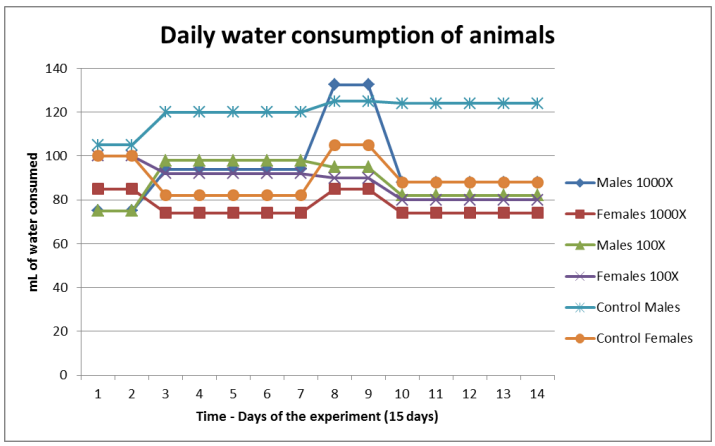

Figure 2: Daily consumption of water in mL of the population of animals for acute toxicity test.

The only significant difference was between the control and 1000X female groups and the 100X and 1000X male groups.

These observations may be due to the weight difference and for leaking water in the cages and not to ^{18}F-FDG toxicity.

5.2.3 Clinical signs, necropsy and histopathological findings

The clinical signs demonstrated by the animals during the experiment were the stress on the day of administration, with agitation in most animals, cries were very common in females and

nose bleeding in only two rats. Half of the rats showed bruising at the injection site, with the passing of days improved, disappearing altogether.

At necropsy, the results were the same for all: severe diffuse pulmonary congestion, severe diffuse splenic congestion; Loads of pasty yellow bowel and stomach; severe diffuse hepatic congestion; moderate bilateral renal congestion, mild diffuse congestion in the Central Nervous System. The only animal that differs is the mouse 5 from the 1000X group instead of presenting severe diffuse pulmonary congestion, showed mild diffuse pulmonary congestion. The data from autopsy indicate the cause as congestion by CO_2, which promoted an increase in volume and blood flow in organs, in order to recover the oxidation thereof by increasing red blood cells [6, 7].

Histological examination complemented the data from the autopsy, showing the blood congestion in all target organs, vacuolar degeneration of hepatocytes due to accumulation of fat or glycogen caused by diet, presence of protein in the renal tubules also due to the diet, two rats showed autolysis tubular kidney.

The results of the autopsy and histological reports were due to the use of CO_2 and not to ^{18}F-FDG injection.

5.3 Subacute Toxicity Studies

5.3.1 Body Weight

The loss and gain of weight was measured during 28 days of experiment, as shown in FIG. 3. The weight measurements were performed on the 1st day of the experiment, after administration and then on days 3, 8, 10, 15, 17, 22, 24 and 28 of the experiment.

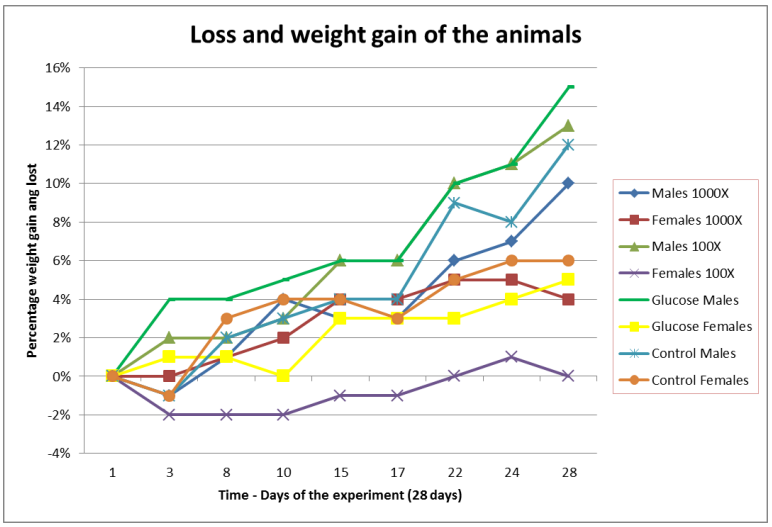

Figure 3: Variation in weight percentage of the control, glucose, 100X and 1000X groups for the subacute toxicity test.

Both females and males groups demonstrated that the weight variation was not statistically significant showing no toxicity related to exposure to ^{18}F-FDG.

5.3.2 Consumption of water and food

The consumption was noted by boxes containing 3 animals in each group. (Table 1)

TABLE 1: Number of rats boxes and their corresponding groups

Box1	Female control group
Box 2	Male control group
Box 3	Glucose female group
Box 4	Glucose male group
Box 5	100X female group
Box 6	100X male group
Box 7	1000X female group
Box 8	1000X male group

In each box was placed a water cooler 900 mL of water, where the days of measurement consumption was measured and evaluated trough and then was added to 900 ml again until the next measurement.

FIG. 4 shows the daily consumption of the boxes. The average of the female boxes were 87.4 mL for the control group, 117.5 mL for the glucose group, 89.4 mL for group 100X and 88.5 mL for group 1000X. The average daily consumption for the males in the control group was 132.7 mL, 116.8 mL for males of the glucose, 125.4 mL for males of the 100X and 102.2 mL for males group of 1000X.

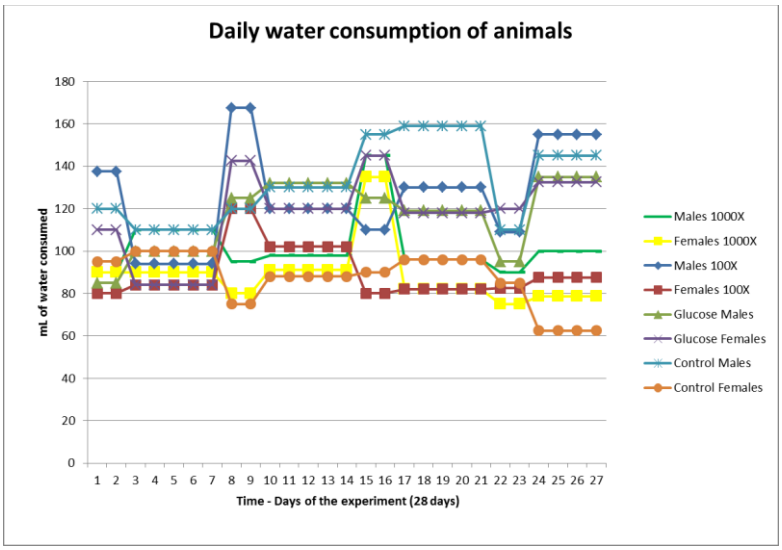

Figure 4: Water consumption in mL daily boxes of populations in the control, glucose, 100X and 1000X groups for the subacute toxicity test.

Only two groups showed statistically difference from the control group, the female glucose group and male 1000X group but not related to ^{18}F-FDG injection.

The Fig.5 shows the daily food intake of the animals. The average consumption of the females groups was 38.4 g for the control group, 39.7 g for group glucose, 39.7 g for group

and 38.5 g of 100X to 1000X group. For males the average consumption for the control group was 60.7 g, 64.8 g for group glucose, 62.5 g for the group of 100X and 56.8 g for group 1000x.

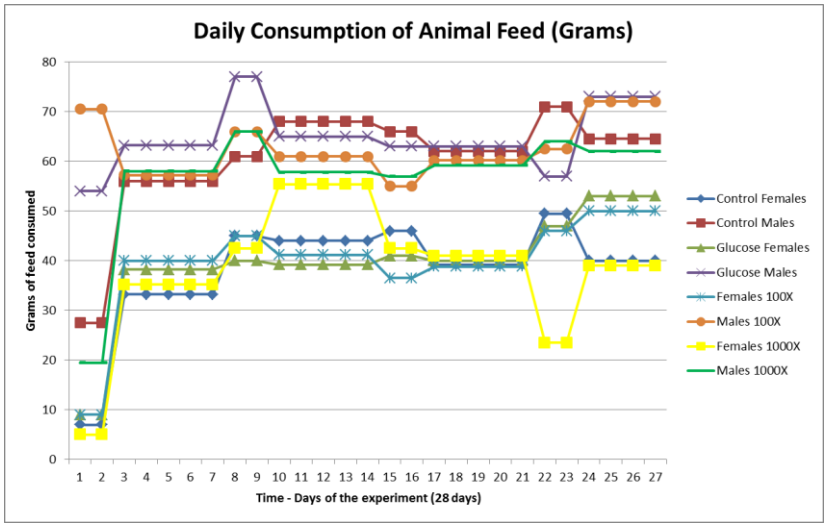

Figure 5: Consumption of food intake in grams of boxes of populations in the control, glucose, 100X and 1000X groups for the subacute toxicity test.

Statistically, the food intake of all groups was not significant different between them.

5.3.3 Clinical signs, necropsy and histopathological findings

Most animals, particularly females showed signs of stress and agitation during and after injection which may have influenced the weight values.

Beside stress, no other clinical signs were observed related to the administration of ^{18}F-FDG during the observation period of 28 days.

All animals were sent to necropsy after his death. External examination of all animals found good nutritional status, pale mucous membranes and a small amount of stool firm in the perineal region.

The findings of the autopsy as hemothorax and foci of blood clot in the heart are directly related to cardiac puncture made before the death of the animal, for blood collection.
The pulmonary congestion and hemorrhage were expected, because of the use of thiopental for anesthesia in rats. The Thiopental causes respiratory depression and decreased gas exchange [6,8].

Outbreaks of mild to moderate erosive enteritis in the jejunum were due to common parasite in mice vivarium, the oxiurídeo, *Syphacia obvelata* found in the cecum and colon of the intestine. Although this is considered a low pathogenic nematode, some intestinal disorders are attributed to this parasite as enteritis, rectal prolapse, fecal impaction and also weight loss and alteration of the immune response. This parasite has no connection with the administration of the radiopharmaceutical ^{18}F-FDG [9,10]. This finding was not statistically significant, when the groups were compared with the control.

The moderate hepatic congestion is another finding normal because when the animal dies the circulating blood remains in the body.

The necropsy showed that no toxicity could be related to the administration of ^{18}F-FDG.

Overall the histopathological findings were not related to the injection of ^{18}F-FDG.

5.3.4 Blood Count, Hepatic function (alanine transaminase-ALT) and Renal Function (Creatinine)

Again the laboratory analysis of blood, ALT and creatinine showed no statistically difference between the animal groups, and no toxicity signs related to ^{18}F-FDG injection.

3. CONCLUSIONS

The acute toxicity study did not show any toxic effect related to the administration of doses of 100 times and 1000 times more the standard dose of the radiopharmaceutical ^{18}F-FDG in rats exposed. The subacute toxicity confirmed the report of acute toxicity.
Even the rats exposed to a greater amount of ^{18}F-FDG for a longer period, showed no significant evidence to classify the ^{18} F-FDG as a toxic substance.

ACKNOWLEDGMENTS

The authors thank CNEN and IPEN for funding the work and for the scholarship.

REFERENCES

1. DIRF- DIRETORIA DE RADIFÁRMACIA. Procedimentos Operacionais - Desenvolvimento de Novos Radiofármacos. Fev. 2010.
2. SITE 1: http://www.sbcc.com.br/revistas_pdfs/ed%2045/30.IPEN.pdf. Acessado 03/10/2012.
3. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Boas Práticas de Fabricação de Radiofármacos e Medicamentos. Dez. 18, 2009. (RDC 63)
4. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Registro de Radiofármacos. Dez. 18, 2009. (RDC 64)
5. MARZIN, D. Preclinical Evaluation of Radiopharmaceutical Toxicological Prerequisites. Nuclear Medicine and Biology, v. 25, p. 733-736, 1998.
6. FISH, R.E.; DANNEMAN, P.J:BROWN, M.;KARAS, A. .Anesthesia and Analgesia in Laboratory Animals. London: Elsevier Inc., 2008.
7. CONLEE, K. M.; STEPHENS, M.L.; ROWAN, A.N.; KING, L.A. Carbon dioxide for eutanásia: concerns regarding pain and distress, with special reference to mice and rats. Review article. 2004.
8. SITE 7: http://www.medicinanet.com.br/bula/5034/thiopentax.htm. Bula Tipental. Acessado em 07/05/2013.
9. DOYLE, R.L.; MONTEIRO, S.G.; GRAÇA, D.L.; SANTURIO, J.M. Avaliação helmintológica de camundongos criados em biotério experimental. Revista FZVA Uruguaiana, v.13, n.2, p.108-115, 2006.

10. SITE 8: http://www.nap.edu/openbook.php?record_id=1429&page=156. Infectious Diseases of Mice and Rats. Acessado em 13/05/2013.