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Article

Influence of radiation on endotoxin test using the PTS™ for 18-FDG radiopharmaceutical

Ralph Santos-Oliveira

Nuclear Engineering Institute, Division of Radiopharmacy, Brazil.

F-18 FDG (2-[18-F] fluoro-2-deoxy-D-glucose) is the most frequently used radiopharmaceutical for PET and PET CT imaging exams. The FDA recently approved the use of the PTS™ (Portable Test System) as an alternative to the standard test proposed by the United States Pharmacopeia using the LAL (Limulus Amebocyte Lysates), that takes longer to perform (about 1h) than the PTS™ (15 min). Recent studies have demonstrated that radiation could interfere with the PTS™ test. In order to study the effects of radiation on the PTS™ test and/or equipment, 27 batches of F-18 FDG produced in the Nuclear Engineering Institute were analyzed. The results showed that no direct correlation with radiation was found in any of the cases.

Uniterms: 18-FDG/utilization. Radiopharmaceuticals. Nuclear medicine. Endotoxin test. Portable Test System/radiation effects

O FDG-18 é o radiofármaco mais utilizado nos exames de PET e PET CT. O FDA recentemente aprovou o uso do PTS™ (Portable Test System) como método alternativo ao teste padrão de endotoxina, proposto pela Farmacopéia Americana, considerando que no primeiro há um tempo de espera de 1 hora frente a somente 15 minutos do segundo. Estudo recentes demonstram que a radiação poderia interferir no teste do PTS™. De modo a avaliar os efeitos da radiação no teste PTS™ foram analisados 27 lotes de F-18 FDG produzidos no Instituto de Engenharia Nuclear. Os resultados demonstraram que em todos os casos nenhuma correlação direta com a radiação foi observada.

Unitermos: FDG-18/utilização. Radiofármacos. Medicina nuclear. Teste de endotoxina. Portable Test System/efeitos da radiação.

INTRODUCTION

Radioisotopes are used in nuclear medicine for diagnostic and therapeutic purposes. Radiopharmaceuticals may be used in oncology at the initial stage, to assess response to treatment, residual disease, recurrent diagnosis or restaging. Another field of study involving radiopharmaceuticals is that of large vessel vasculitis, granulomatous diseases and dementia (Santos-Oliveira, Carneiro-Leão, Smith, 2008).

The word pyrogen, which can be traced to the Greek pyro, meaning burning or fire and gennaó meaning to make or to create, is now used as an apt description for substances that produce elevated body temperature. Pyrogens are usually bacterial products and remains, or decaying products of bacterial cell walls. Even at minimum doses, these substances induce elevated body temperature when injected into humans and animals. Pyrogens are usually high-molecular-weight substances of a polymerous nature, akin to lipopolysaccharids. Pyrogens can be either microbial or non-microbial. Endotoxins are high-molecular-weight complexes associated with the outer membrane of gram-negative bacteria (GNB). They are the most usual cause of the elevated body temperature, induced by contaminated drug products. Their pyrogenic activity is higher than that of other pyrogenic substances. It could be said that the absence of such bacterial endotoxins in a drug implies the absence of pyrogenic components in the studied drug in general. Endotoxins are similar to lipopolysaccharids, they are heat stable and can survive the

***Correspondence:** R. Santos-Oliveira. Nuclear Engineering Institute, Division of Radiopharmacy. Rua Hélio de Almeida no. 75 -Ilha do Fundão, 21.941-906 - Rio de Janeiro - RJ, Brazil. Phone: 55 21 2173-3758. Fax: 55 21 2173-3782. Email: roliveira@ien.gov.br

sterilization process (Blechová, Pivodová, 2001).

The use of LAL for endotoxin detection stemmed from Bang's observation that the infection of *Limulus polyphemus*, the horseshoe crab, induced by GNB, results in extensive intravascular clotting and death. Levin and Bang later demonstrated that the extracellular coagulation of *Limulus* hemolymph (blood) was caused by a reaction between endoxin and a coagulative protein in amebocytes, circulating in Limulus hemolymph (Levin, Bang 1964). In a subsequent study, Levin et al. developed a sensitive assay for endotoxin in human plasma using the material lysed from Limulus amebocytes (Levin, Tomasulo, Oser, 1970). Young, Levin and Prendergast then isolated, purified and described the LAL coagulative protein and proved that the reaction between lysate and endotoxin is of an enzymatic nature (Young , Levin, Prenderagst, 1993).

Limulus Amebocyte Lysate (LAL) is a lyophilized preparation made from the amebocytes of the horseshoe crab Limulus polyphenus. The LAL will clot to form a gel in the presence of endotoxin from gram-negative microorganisms. Since the LAL test method is far more rapid than the USP (United States Pharmacopoeia) rabbit pyrogen test method, it has been used as a preferable test (Watchel, Tsuji, 1977). Nevertheless, despite being more rapid than the rabbit test method, the LAL test is still slow, especially for radiopharmaceuticals.

The PTS™ (Portable Test System™) is a novel endotoxin assay in which dry LAL-reagents are fixed to a cartridge and results are interpolated from an archived standard curve by a validated chromogenic assay. The use of the of PTS has become an alternative to the traditional USP test using LAL due the shorter time taken to complete the whole process, about 15 minutes against 1 hour for the traditional test (FDA, 1987; Zijlstra *et al.*, 1997; Fukumori *et al.*, 2007)

However, studies by Hung, Iverson, Jacobson and Mahoney (2005) demonstrated that special care is necessary when using the PTS with radiopharmaceuticals, since during the test the FDG can inhibit gel formation. Therefore, in order to prove this theory an experimental study with 27 samples of 18-FDG produced in the Nuclear Engineering Institute was conducted.

METHODOLOGY

The assays were performed using the Endosafe PTS™ equipment and the kit for the test (cartridge) from the Charles River Laboratories™ . The methodology adopted was that described in the equipment manual (25µL of F-18 FDG injectable solution were fixed in the cartridge and then the equipment run). The samples used were 27 successive daily batches of F-18 FDG produced routinely in the Nuclear Engineering Institute. No dilution factors were used.

RESULTS AND DISCUSSION

The PTS™ works with a dry LAL reagent and uses validated interpolated internal values. Among the parameters revealed by the PTS™ test, the most important is the recovery percentage. Briefly, the recovery rate shows the ability of the sample to react with the dry LAL reagents and form the chromogenic compound. According to the manufacturer of the PTS™, a good range for the recovery parameter is between 50% and 200%. Values above or below this range mean that the reaction between the sam-

TABLE I - Activity and correlation with other parameters

RHP/ Sample	Dilution Factor	PTS™ Result (EU/ml)	PTS™ Recovery test	Activity (µCi)
FDG-18/1	1	<0.050	74	477.0
FDG-18/2	1	<0.050	15	960.0
FDG-18/3	1	<0.050	104	811.0
FDG-18/4	1	0.056	90	923.0
FDG-18/5	1	0.118	146	972.0
FDG-18/6	1	0,164	102	972.0
FDG-18/7	1	<0.050	94	772.0
FDG-18/8	1	<0.050	110	865.0
FDG-18/9	1	<0.050	92	865.0
FDG-18/10	1	<0.050	76	846.0
FDG-18/11	1	<0.050	38	902.0
FDG-18/12	1	<0.050	57	852.0
FDG-18/13	1	<0.050	40	751.0
FDG-18/14	1	<0.050	52	530.0
FDG-18/15	1	<0.050	74	701.0
FDG-18/16	1	<0.050	62	630.0
FDG-18/17	1	<0.050	47	505.0
FDG-18/18	1	<0.050	58	739.0
FDG-18/19	1	<0.050	58	652.0
FDG-18/20	1	<0.050	80	800.0
FDG-18/21	1	<0.050	27	1006.0
FDG-18/22	1	<0.050	95	1080.0
FDG-18/23	1	<0.050	44	1149.0
FDG-18/24	1	<0.050	58	1108.0
FDG-18/25	1	<0.050	36	1075.0
FDG-18/26	1	<0.050	100	1298.0
FDG-18/27	1	<0.050	47	1826.0

ple and the reagents of the kit was not good and that the results are not reliable.

Samples 4, 5, 6, are the most representative cases. Variations were detected in the PTS™ test in all these samples, as shown in Table 1. However the values found are under the acceptable limit (0.25EU/mL). Thus, although they are different from the others this does not mean they are not good enough. Relatively to recovery parameter no single observation is possible. The same occurs with the activity, since they are too similar and the results are too different.

In the case of samples 2, 11, 13, 17, 21 and 23, the recovery range value given by the PTS™ was lower than the acceptable limit (50 to 200%), but the endotoxin limit was acceptable at less than 0.050 EU/mL. Although the samples had been approved for the assay, the recovery value is an important parameter linked to the reliability of the results. This means that in a case where the samples have to be re-analyzed the new results found can differ to the original results. Again, no relationship with activity, recovery test and endotoxin limit is possible. The range of each parameter is too high to try to create a hypothesis.

The attempt to correlate all the problems described previously with the radiation of the sample failed, as shown in Table1. Samples 24, 25, 26 and 27 are the most representative. The four samples have the highest values of activity. However, the value of recovery varies without any correlation. For samples where the recovery of the PTS™ test was in the range (50-200%), no relationship with the radiation level was ascertained since no relationship among the values was found.

It is important to note that all these samples were analyzed by the traditional method described in the USP and no clots were formed in any of the cases. This implies that according to the traditional methods all the samples were approved.

CONCLUSION

The PTS was shown to be a good alternative endotoxin test, especially for radiopharmaceuticals, where time is one of the most important parameters during the routine assay. The results were able to corroborate the use of PTS™ in the daily routine for 18-FDG. However, several points must be evaluated in more depth, particularly recovery range.

Although the results shown above demonstrated no relationship between radiation and the endotoxin test using the PTS™ further studies are needed for other radiopharmaceuticals, in particular radiopharmaceuticals based on Iodine since iodine has coloration when in solution and could interfere with the test through the formation of color-complexes.

Therefore, we recommend that before introducing PTS in the daily routine for radiopharmaceuticals, further validations should be conducted.

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