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NOTE

Stabilization of Tc-99m radiopharmaceuticals by chemical additives

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The reliability and applicability of the preparation of the three, for nuclear medicine very important, ^{99m}Tc-radiopharmaceuticals from the inactive (technetium-cold) kit solutions were tested. Each examined commercial kit was dissolved in saline (0.9 % NaCl). The conditions of the storage of the inactive kit solutions till labeling were examined. The main problem is the stablity of the reductant stannous ions which is very difficult to predict. To stabilize and ensure a good quality of the labeled radiopharmaceuticals, ascorbic or gentisic acid were added. It was found that the best results were obtained by keeping the samples frozen at –20 ºC. Both stabilizers can be used but for an effective protection much lower concentrations of ascorbic acid are needed. Its concentrations of 12–60 μg/ml of the kit, stabilized dimercaptosuccinate (DMS) and pyrophosphate (PyP) for about 7–8 days. The solution of 2,3-dicarboxypropane-1,1-diphosphonate (DPD) was found to be stable even without the stabilizer. This could be attributed probably to the chemical nature of this complex. However, in routine praxis the applied procedure demands great care and personel very experienced in radiopharmacy.

Keywords: Radiopharmaceuticals, technetium-99m, dimercaptosuccinate, dicarboxypropane diphosphonate, pyrophosphate.

INTRODUCTION

To make a short-lived radioisotope available at any site in the word, regardless of the distance from the production center, is a rather complicated task. In the case of 6-hour ^{99m}Tc two main innovations enabled the construction of an adequate framework. The first was the development of ^{99}Mo/^{99m}Tc generators. In short, the concept of a radionuclide generator is based on the effective separation of a decaying, longer-lived parent and the daughter radionuclide of shorter half-life so that the daughter is obtained in a pure radionuclidic and radiochemical form. The most common is the chromatographic generator based on fission-produced ^{99}Mo. Separation of ^{99m}Tc from the lon-

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ger lived ^{99}Mo by elution with saline, is performed by the end-user, often very far from the production center. Several reviews cover different types and aspects of ^{99}Mo/^{99m}Tc generators.[1] The introduction of the evacuated vial technique of elution made a very important contribution to generator technology. It eliminated the exposure of the column to water and its consequent radiolysis and limited the possiblity of microbial contamination. Thus, a fresh daily supply of sterile, pyrogen-free eluate ^{99m}Tc is ensured.

The second milestone was the development of ^{99m}Tc-radiopharmaceuticals by the method which is now known also as "instant kit" preparation. It appeared in the literature for the first time in 1971.[2] Althought the basic chemistry was poorly understood at that time, the first generation of radiopharmaceuticals, targeting specific organs and tissues in an organism, was soon developed. Generally, technetium previously reduced by stannous ions to lower valence states, reacts with donor ligands. The ligands coordinate the metal forming complexes which target specific organs and tissues in the human body. The modern instant kit comprises stannous ions, ligands, additives, buffers, *etc*, in lyophilised form in a glass vial under vacuum or in a nitrogen atmosphere. The shelf-life of such a kit, determined experimentally, is usually up to several months under certain storage conditions. Labeling is performed by addition of ^{99m}Tc, obtained by elution of the generator. This procedure is also called the reconstitution of the kit.

Decristiforo *et al.*[3] investigated the applicability of the procedure for the cost effective use of several kits. Instead in ^{99m}Tc eluate, the lyophylized content of the kit vial was dissolved (reconstituted) in pure saline. The technetium-cold (inactive) solution was then kept at low temperature and labeled on demand.

This approach was preliminary tested on freshly prepared solutions of pyrophosphate.[4] In this work the investigations were extended to three important commercial kits in order to determine the reliability of the proposed method for its possible routine clinical application. Two osteotropic preparations, pyrophosphate (PyP) and 2,3-dicarboxypropane-1,1-diphosphonate (DPD), as well as dimercaptosuccinate (DMS) which is, after labeling with ^{99m}Tc, used for the visualization of kidneys, were examined. Ascorbic and gentisic acid were tested as stabilizers. In the experiments, different amounts of the stabilizers were added to the kit solutions and the content of ^{99m}Tc pertechnetate was determined in dependance on time and temperature of storage of the reconstituted kit.

EXPERIMENTAL

The DMS and PyP kits were prepared from commercially purchased chemicals. DPD was laboratory - synthesized.[5] Technetium-99m was obtained by elution of a ^{99}Mo/^{99m}Tc generator. The kits and the generator are commercially produced in the Laboratory for Radioisotopes (Vinča Institute of Nuclear Sciences). Stannous chloride dihydrate (Merck), ascorbic (Merck) and gentisic acid (Fluka) were commercial p.a. grade chemicals.

The "cold" kits were reconstituted by addition of a given volume of the home-made saline solution, divided into 2 ml-fractions and kept either at –20 ºC or at 5 ºC. After definite time intervals, the fractions were thawed and labeled by addition of 0.2–0.3 ml of ^{99m}Tc eluate (30–74 MBq ^{99m}Tc).

The experiments were performed on the following samples:

a) 2-ml fractions of the kits, without any stabilizer, kept either at –20 ºC or at 5 ºC

b) 2-ml fractions of the kits containing either 12 or 60 μg of ascorbic acid per ml, kept either at –20 ºC or at 5 ºC

c) 2-ml fractions of the kits containing either 100, 200 or 500 μg of gentisic acid per ml, kept either at –20 ºC or at 5 ºC.

The content of ^{99m}Tc pertechnetate was determined by ascending paper chromatography on Whatman No. 1 paper with acetone as the mobile phase. ^{99m}Tc-pertechnetate migrates with the solvent front (R_f = 1) while the labeled preparation and reduced, hydrolyzed ^{99m}Tc remain at the start (R_f = 0). The paper was cut into 1-cm pieces and counted in a well-type gamma counter (Gamma 333, ICN, Belgium).

RESULTS AND DISCUSSION

The lyophilized content of the kit vial was reconstituted (dissolved) in ^{99m}Tc eluate. The obtained solution was then divided into a certain number of individual doses for the patients. It happens commonly in routine praxis that at least a part of the ^{99m}Tc kit remains unused and has to be rejected. The proposed procedure[3] should avoid these losses and ensure a cost effective use of the kits. The basic difference, in comparison to the usual procedure, is that the content of the vial is dissolved in pure saline instead in ^{99m}Tc eluate. A fraction of technetium-cold solution (without ^{99m}Tc) which will not be used is separated and labeled later on demand.

It is important to ensure that ^{99m}Tc radiopharmaceuticals of adequate quality are obtained. Therefore, appropriate storage conditions should be determined, as well as the time span during which such a kit solution can be reliably used.

This approach was first tested on freshly prepared solutions of pyrophosphate.[4] In the experiments, presented in this paper, the proposed method was applied to the solutions obtained by the reconstitution of the three commercially available kits: DMS, DPD and PyP.

On the basis of previous results, it was concluded that storage of the samples at low temperature as the only protection can not ensure a stable quality of the labeled preparation. The content of ^{99m}Tc-pertechnetate rises, thus lowering the required radiochemical purity of the kit. The presence of some chemicals as stabilizers was found to be necessary. So, in the present experiments, the kit samples contained either ascorbic or gentisic acid as chemical stabilizers.

In principle, labeling efficiency of radiopharmaceuticals depends on several factors, such as the concentration of the ligand and stannous ions, molar ratio ligand/reductant, pH and time of reaction. The composition of the solutions of the kits used in the experiments and in the major fields of application of ^{99m}Tc(Sn)-DMS, ^{99m}Tc(Sn)-DPD and ^{99m}Tc(Sn)-PyP are presented in Table I.

As an example of the stabilization pattern, Table II shows the results obtained with the dispensed solutions of PyP. The content of ^{99m}Tc-pertechnetate in dependence on the concentration of ascorbic acid, as well as on the temperature and time of storage are given.

It is arbitrarily taken that a kit is considered as stabilized if the content of ^{99m}Tc-pertechnetate does not exceed 5 %.

TABLE I. Composition of the solutions of DMS, DPD and PyP and the fields of application of ^{99m}Tc-labeled radiopharmaceuticals

Kit	Volume of the "cold" kit/ml	Ligand/ μmol/ml	Reductant/μmol $SnCl_2$/ml	L/R	pH	Applications of ^{99m}Tc radiopharmaceuticals	Ref.
DMS	8	1.37	0.445	3.1	3–4	Renal scintigraphy; measurement of renal fixation; determination of kidney function	6
DPD	10	3.74	0.223	16.8	7–8	Skeletal scintigraphy	5
PyP	6	22.4	0.89	25.2	5–6	Bone and joint scintigraphy	7

According to the data presented in Table II, it can be seen that already the lowest examined concentration of ascorbic acid (12 μg/ml) is sufficient to keep the content of ^{99m}Tc-pertechnetate far below 5 % for five days in the samples stored at a low temperature (–20 ºC).

TABLE II. Content of free ^{99m}Tc-pertechnetate in the dispensed ^{99m}Tc(Sn)-PyP in dependence on the concentration of ascorbic acid, temperature and time of storage

Storage temperature	–20 ºC		5 ºC	
Time of storage days	Content of asc. acid/μg/ml	Content of $^{99m}TcO_4^-$/%	Content of asc. acid/μg/ml	Content of $^{99m}TcO_4^-$ /%
1	0	2.8±0.3	0	3.5±0.2
	12	0.2±0.1	12	0.6±0.2
	60	0.2±0.1	60	0.6±0.3
5	0	4.5±3	0	–
	12	0.2±0.1	12	–
	60	0.3±0.1	60	–
7	0	–	0	9.3±1.3
	12	0.3±0.1	12	0.2±0.1

The investigations show that both ascorbic and gentisic acid can be used as chemical stabilizers. Generally, there is no substantial difference in the mode of stabilization. Therefore, the results obtained by using gentisic acid are not shown separately.

The effect of ascorbic acid on the stabilization of DMS, PDP and PyP in dependence on its concentration, as well as on the time and temperature of storage is shown in Table III, in which the concentrations and time during which the radiopharmaceutical is stabilized, *i.e.,* the content of ^{99m}Tc is 5 % or less, are given.

The content of free ^{99m}Tc-pertechnetate in the DMS and PyP depends on the concentration of the stabilizer. However, the desired degree of protection is achieved with much lower concentrations of ascorbic (12–60 μg/ml) than gentisic acid. To achieve a similar effect much higher concentrations of gentisic acid have to be used. Up to 100–200 μg/ml of gentisic acid stabilizes DMS for only about 2 days storage at –20 ºC

but for longer storage times even much higher concentrations of gentisic acid (500 μg/ml) were found to have no effect. This high concentration of the stabilizer results in colorization of the kit solution. The possible negative effect on the biodistribution of the labeled kit would also have to be tested. This finding also has practical meaning–ascorbic acid is a better chemical stabilizer and is also less expensive than gentisic acid.

The results presented in Table III indicate that the stability of kit solution samples depends both on the content of ascorbic acid and on the storage conditions. The lower storage temperature (–20 ºC) is favourable. Together with the use of ascorbic acid it enables the production of labeled radiopharmaceuticals DMS and PyP, which are stable up to about 7–8 days after dispensing of the inactive solution.

TABLE III. Time of stabilization (days) of DMS, DPD and PyP in dependence on the concentration of ascorbic acid and temperature of storage

Storage temperature		– 20 ºC		5 ºC	
Kit	Content of asc. acid/μg/ml	Time of stabilization/days	Content of $^{99m}TcO_4^-$/ %	Time of stabilization/days	Content of $^{99m}TcO_4^-$ /%
DMS	0	1	4.50±0.5	1	≤ 1
	12	1	≤ 1	2	1.8±0.4
	60	7	≤ 1	2	≤ 1
DPD	0	7	≤ 1	5	≤ 1
	12	7	≤ 1	5	≤ 1
PyP	0	1	2.8±0.3	1	3.5±0.2
	12	8	≤ 1	7	≤ 1

However, by analysing the data given in Table III it can be concluded that the stability of the kit solutions depends also on some other factors. Generally, the main problem which occurs is the stability of stannous ions which is difficult to predict. The nature of the prepared complex should also be taken into account. The DPD kit has the highest stability among the examined kits and although it contains the smallest concentration of the reductant Sn(II), it is stable even without ascorbic acid.

CONCLUSIONS

The reliability of the use of fractionated technetium-cold kits in the preparation of the three ^{99m}Tc radiopharmaceuticals was tested. Three important kits for application in nuclear medicine, DMS, DPD and PyP, were examined. The obtained results show that the cold kit should be stored at low temperatures (–20 ºC). Ascorbic and gentisic acids were applied as chemical stabilizers. Ascorbic acid was found to be more suitable as much lower concentrations were needed to achieve the desired stability. In the cases of DMS and PyP, about 12–60 μg/ml of ascorbic acid stabilize the kits for 7—8 days after dispensing. DPD was found to be highly stable even without a stabilizer. The results show that this method could be a promising possibility for a cost effective use of

^{99m}Tc-radiopharmaceuticals. However, they also illustrate the difficulties which could arise in routine clinical practice. It is very difficult to predict the stability of stannous ions, as it depends also on the nature of the ligand, the presence of other components, differences in the production batches, *etc*. Therefore it seems that the proposed method can only be applied with great care and by people experienced in radiopharmacy.

И З В О Д

СТАБИЛИЗАЦИЈА Tc-99m РАДИОФАРМАЦЕУТИКА ХЕМИЈСКИМ АДИТИВИМА

ЈУРИЈ ВУЧИНА и НАДЕЖДА ВУКИЋЕВИЋ

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Испитан је поступак ефикасног и економичног искоришћења три комерцијална, за нуклеарну медицину врло важна ^{99m}Tc радиофармацеутика. Он се, за разлику од стандардног (растварање у елуату ^{99m}Tc), заснива на растварању лиофилизованог садржаја бочице са препаратом у неактивном, чистом физиолошком раствору 0,9% NaCl. У раду одређени су услови и време чувања оваквих раствора који обезбеђују да се, по обележавању ^{99m}Tc, добију радиофармацеутици одговарајуће радиохемијске чистоће. Раствори препарата, са или без антиоксиданса аскорбинске или 2,5-дихидрокси-бензоеве киселине, до обележавања чувају се на сниженој температури. Оба хемијска стабилизатора могу се применити, али су за постизање добрих резултата потребне знатно ниже концентрације аскорбинске киселине. Њене концентрације од 12–60 μg/ml довољне су за стабилизацију димеркапто-сукцината (DMS) и пирофосфата (PyP) током 7–8 дана од разливања. Нађено је да је 2,3-дихидрокси-1,1-пропандифосфонат (DPD) стабилан и без додатка стабилизатора. То се може објаснити природом комплекса. Међутим, недостатак овог поступка за рутинску примену је у томе што његова поузданост не зависи само од концентрације реактаната и услова чувања већ и од природе лиганда и других фактора.

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