The Journal of NUCLEAR MEDICINE

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J Nucl Med. 2010;51:495-496.
Published online: February 11, 2010.
Doi: 10.2967/jnumed.109.071084

The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

SNMMI SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING

Impurity in ^{68}Ga-Peptide Preparation Using Processed Generator Eluate

TO THE EDITOR: Zhernosekov et al. (*1*) have published a method for processing generator eluate from ^{68}Ge/^{68}Ga generators for medical applications, and currently this method is widely used for clinical preparations, especially of ^{68}Ga-labeled 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-D-Phe^{1},Tyr^{3}-octreotide (DOTATOC) and other somatostatin analogs. The method is based on preconcentration of ^{68}Ga ions in the eluate on a cation exchanger followed by selective washing and subsequent elution using hydrochloric acid/acetone solutions. This method removes unwanted metallic impurities and reduces the volume, as is especially suitable for radiolabeling of small peptides such as DOTATOC. We investigated this method in an attempt towards developing a fully automated system. During our validation steps of this method, we observed an unknown peak in the ultraviolet trace (220 nm) of the high-performance liquid chromatograms of ^{68}Ga-labeled peptides increasing over time with prolonged storage of the hydrochloric acid/acetone solutions. The peak eluted closely to DOTATOC or DOTA-D-Phe^{1},Tyr^{3}-octreotate used for radiolabeling when analyzed under standard acetonitrile/0.1% trifluoroacetic acid/water gradient high-performance liquid chromatography (HPLC) conditions. By means of gas chromatography mass spectrometry, we finally identified the peak to be 4-methyl-3-penten-2-on (mesityloxide), a condensation product of acetone. Apparently, this impurity and the ^{68}Ga-labeled peptide have comparable properties on the reversed-phase (C-18) material used both for purification after synthesis and for quality control by HPLC. Because of the high boiling point (129°C) of this compound, the peak was detected even when prolonged heating was applied, and column purification failed to remove it. We could show that the amount of mesityloxide formed is dependent on storage time and storage conditions of the hydrochloric acid/acetone stock solutions. Storage at room temperature and exposure to light resulted in formation of milligram amounts, and up to 0.7 mg was detected in a final volume corresponding to a patient preparation. If the mixtures were prepared freshly or stored with protection from light at −20°C, this amount was below 10 μg. Mesityloxide is not a highly toxic compound, and median lethal dose in rats (1.12 mg/kg (*2*)) is comparable to that of acetone. However, as no limits for parenteral preparations are available in relevant documents such as the U.S. Pharmacopeia, the amount should be limited; a reasonably achievable limit seems to be 50 μg per maximum volume injected. We strongly recommend all users of this method to include into their quality control protocols a quantification of this impurity by means of HPLC–ultraviolet detection or gas chromatography. Additionally, careful validation of preparation and storage of hydrochloric acid/acetone solutions has to be performed. Alternative preparation methods avoiding the use of hydrochloric acid/acetone mixtures have been described (*3,4*) and may also be envisaged.

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DOI: 10.2967/jnumed.109.070953

REPLY: I thank Dr. Petrik and colleagues for their interest in our work (*1,2*). Our method of processing ^{68}Ga eluates indeed has several advantages allowing instant kit-type synthesis procedures. First, it purifies this generator-derived positron emitter of metals such as zinc, iron, and titanium, significantly improving the yield of subsequent labeling reactions. Second, it lowers the initial breakthrough of ^{68}Ge by more than 4 orders of magnitude down to negligible levels, thus minimizing concerns about radiopharmaceutical contamination by this long-lived radionuclide. Third, it reduces the total volume of the purified ^{68}Ga fraction to 0.4 mL, and finally, it removes excess hydrochloric acid originating from the generator eluate. Recent improvements also demonstrate that the intermediate adsorption of purified ^{68}Ga on the small cation exchange cartridge opens the door toward synthesis of lipophilic ^{68}Ga-compounds in nonaqueous solvents (*3*).

Moreover, the removal of ^{68}Ge represents an inherent safety procedure, as it automatically eliminates any unexpected leakage from the generator. Such a sudden dramatic problem, eventually induced by defects and incorrect handling of the generator, is neither directly seen nor avoided by using fractionation as an approach toward eluate postprocessing (*4,5*).

Consequently, postprocessing ^{68}Ga eluates (*1,2*) has become a well-adopted procedure, applicable both to 0.1N and to 0.6N HCl eluate concentrations (for TiO_2- and SnO_2-based generators, respectively).

In the cation exchange-based processing chemistry and subsequent (for example) ^{68}Ga-DOTA-octreotide synthesis and product purification steps, nontoxic ingredients such as Millipore water, saline, acetone, and ethanol are involved exclusively. Preparation and storage of these solvents and acetone/HCl mixtures in the context of application of ^{68}Ga-labeled radiopharmaceuticals for patient diagnoses is well defined as the responsibility of the

qualified persons in installation qualification, operational qualification, and standard operating procedure protocols.

Consequently, the solutions have to be prepared from high-purity batches, have to be kept in closed vials, and have to be refrigerated or stored at −20°C for a limited time only. If not, the sterility of the Millipore water and the saline solutions is not guaranteed, the composition of the acetone/HCl mixtures will change because of the low boiling points of the compounds, and the acetone in acidic media will undergo an aldol addition reaction forming 4-methyl-3-penten-2-on. We appreciate the effort of Petrik et al. to "finally" identify this well-known product, confirming the standard education of chemistry students (*6*).

If it was the intention of the present letter to the editor to reflect the relevancy of creating and following standard operating procedures for the synthesis of radiopharmaceuticals, we completely agree. Regarding the nontoxic compound 4-methyl-3-penten-2-on, Petrik et al. correctly state that its formation is negligible if acetone/HCl mixtures are stored with protection from light at −20°C or if freshly prepared mixtures are used.

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DOI: 10.2967/jnumed.109.071084

Side Effects Profile in Humans of ^{11}C-(+)-PHNO, a Dopamine $D_{2/3}$ Agonist Ligand for PET

TO THE EDITOR: ^{11}C-(+)-4-propyl-9-hydroxynaphthoxazine ((+)-PHNO) is a new PET ligand developed by our group. Binding assays show that (+)-PHNO displays high affinity and selectivity for the D_2 receptor (*1*). Recently, it has been noted that ^{11}C-(+)-PHNO has a preferential affinity and selectivity in vivo for the D_3 receptors (*2*). Because ^{11}C-(+)-PHNO is an agonist radiotracer for D_2 and D_3, it is likely to produce pharmacologic effects, in contrast to antagonist radiotracers. We reviewed all ^{11}C-(+)-PHNO consecutive scans obtained in our PET center for side effects. Mass injected (μg), subjects' weight (kg), and dose (μg/kg) were included in the analysis. Side effects were recorded on the basis of the subjects' self-report either during or right after finalization of the scan. A physician was available at all times to confirm and treat any possible side effects. Side effects were coded as 0 (no effect), 1 (nausea), or 2 (vomiting), based on our early experience with ^{11}C-(+)-PHNO (*3*). Odds ratios (ORs) were calculated using logistic regression analyses to investigate the relationship between dose, mass, and effects.

The number of reviewed ^{11}C-(+)-PHNO scans totalled 486. Injected mass ranged from 0.85 to 5.56 μg, with a mean of 2.30 μg (SE, 0.024 μg). Injected doses ranged from 0.01 to 0.08 μg/kg, with a mean of 0.03 μg/kg (SE, 0.0004 μg/kg). No effect was present in 84.6% of the scans reviewed; nausea was present in 14.3%, and vomiting in 1.1%. Symptoms arose 3–5 min after the injection and subsided within 7–12 min in all cases. In none of the cases was any medical action required.

In a logistic regression model including all subjects, nausea was significantly predicted by dose (Wald = 21.70, $P < 0.001$, OR 1.99) and mass (Wald = 16.319, $P < 0.001$, OR = 2.826), and vomiting was significantly predicted by dose (Wald = 7.31, $P < 0.007$, OR = 2.66) but not by mass injected (Wald = 0.694, $P = 0.405$, OR = 1.810). When only drug-free volunteers were analyzed ($n = 209$), no effect was present in 79.8% of the cases, nausea was present in 18.7%, and vomiting in 1.5%. In a logistic regression model including only drug-free volunteers, nausea was significantly predicted by dose (Wald = 6.98, $P < 0.008$, OR 1.54) and mass (Wald = 11.981, $P = 0.001$, OR = 2.843). Vomiting was predicted at a trend level by dose (Wald = 3.33, $P < 0.06$, OR 2) but not by mass (Wald = 0.105, $P = 0.746$, OR = 1.303). When only antipsychotic-treated participants were analyzed ($n = 66$), no effect was present in 97% of the cases, nausea was present in 3%, and no vomiting was present in any. In a logistic regression model including only these subjects, nausea was significantly predicted neither by dose (Wald = 2.25, $P < 0.13$) nor by mass (Wald = 0.000, $P = 0.99$). In all cases, when an injected dose of 0.029 μg/kg or less was selected, there was no relationship between dose and nausea.

The side effects reported in this study are consistent with the expected agonism at the D_2- and D_3-receptor (*4–7*).

We conclude that doses of ^{11}C-(+)-PHNO of 0.029 μg/kg or less are highly unlikely to produce any side effects in humans and that ^{11}C-(+)-PHNO is a safe agonist radiotracer for PET in human studies of health and disease.

ACKNOWLEDGMENTS

We acknowledge the scientists and technicians from the PET center who carried out the ^{11}C-(+)-PHNO scans. This work is supported in part by the CIHR and the OMHF New Investigator Awards.

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