TECHNICAL NOTE

Radiopharmaceutical Factors in the Variable Quality of [^{99m}Tc]HM-PAO Images of the Brain

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Variability in the quality of [^{99m}Tc]HM-PAO images of the brain has been attributed to differences between kits and the addition of excessive amounts of pertechnetate to the kits. A retrospective study showed no significant differences between batches of kits with respect to radiochemical purity (RCP) or image quality. Up to 3000 MBq (81 mCi) pertechnetate could be added to the kit without adversely affecting RCP or image quality. It was shown that image quality was directly affected by RCP at time of injection and dropped sharply when RCP fell below 85%. Interstitial injection and mixing with blood prior to injection also resulted in poor image quality. Pretreatment with perchlorate reduced uptake of activity in the parotid glands; this improved image quality and reduced the influence of RCP.

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Technetium-99m hexamethyl propyleneamine oxime ([^{99m}Tc]HM-PAO) is a recently introduced radiopharmaceutical for cerebral blood flow imaging with single photon emission computed tomography (SPECT) (*1*). Its mechanism of retention in the brain and other tissues involves rapid transformation into a non-diffusible species, possibly involving interaction with glutathione (*2,3*).

The [^{99m}Tc]HM-PAO complex is inherently unstable (*1*). Although this instability is necessary for trapping in the brain, the in vitro instability of [^{99m}Tc]HM-PAO makes it inconvenient to use; its shelf-life after reconstitution is only 30 min (*4*). In addition, the HM-PAO kit contains a very low level of stannous chloride, 7.5 μg (*1,4*), which makes the labeling efficiency sensitive to the amount of pertechnetate added and the quality of the pertechnetate (i.e., the ^{99}Tc/^{99m}Tc ratio [specific activity] and amount of radiolytic products present). These quality factors are in turn a function of the time since elution of the generator and the time since previous elution of the generator (in-growth or buildup factor).

Because of these factors, the manufacturer recommends that HM-PAO kits be reconstituted with a maximum of 1110 MBq (30 mCi) pertechnetate which was eluted <4 hr earlier from a generator which had been eluted <24 hr previously. Furthermore, [^{99m}Tc]HM-PAO must be injected within 30 min of reconstitution (*1,4*).

However, the high cost of HM-PAO kits and lack of adequate third-party reimbursement make use of the single-dose vial impractical. Many users are exceeding the activity limit in order to obtain more than one dose per vial. One recent comparison costed HM-PAO at two doses per vial (*5*).

SPECT images of the brain following i.v. injection of [^{99m}Tc]HM-PAO are degraded by variable amounts of extracerebral activity, particularly in the parotid glands. The nature of this activity in the parotid glands is not certain; however, if activity is present as pertechnetate, we would also expect that pertechnetate would accumulate in the choroid plexus, resulting in image degradation. The variability in the amount of extracerebral activity has been attributed to exceeding the manufacturer's specifications, vial-to-vial variation, and batch-to-batch variation in tin content and isomeric purity.

We conducted a retrospective analysis of radiopharmaceutical parameters in the preparation of [^{99m}Tc] HM-PAO. This resulted in several observations which were then tested prospectively.

METHODS

Technetium-99m HM-PAO was prepared by adding 1100–3500 MBq (30–95 mCi) [^{99m}Tc]pertechnetate (^{99}Mo/^{99m}Tc generator from Nordion International Inc., Kanata, ON) in 2–5 ml saline to freeze-dried kits ("Ceretec", Amersham Canada Ltd., Oakville, ON). On each occasion, a record was kept of the age of the generator eluate and the time since previous elution of the generator. Five min after reconstitution, radiochemical purity (RCP) was determined either by the recommended 3-system chromatographic method (*1,4*) or by a rapid extraction technique validated in our laboratory (*6*). The in vitro stability of [^{99m}Tc]HM-PAO was determined by repeated

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measurements of RCP and the decline in RCP with time was fitted to a monoexponential function by the method of least squares to calculate the decomposition rate constant, k_d (*7,8*).

Patients referred for cerebral perfusion imaging received ~740 MBq (~20 mCi) [^{99m}Tc]HM-PAO by i.v. injection (one to three doses per vial). Starting 5–120 min after injection, 64 images of 30 sec each were obtained over 360° with a rotating gamma camera (Siemens ZLC 3700) interfaced to a computer (MDS A^3). Data were smoothed with a prefilter, then reconstructed with a Hanning filter into a 64 × 64 matrix. For assessment of image quality, in the planar left lateral view from raw projection data the count density was calculated in regions of interest placed over the basal structures (basal ganglia and temporal lobes), frontal cortex, parotid glands, and nasal region. Because the count density in the basal structures was always greater than that in the cortex, this was used as a reference area for target activity. Ratios of activity in the background areas (i.e., parotid and nasal regions) to activity in the basal structures were calculated to reduce patient-to-patient variability in count densities. The parotid/basal structure ratios were of particular interest as we wished to observe the effect of perchlorate on this ratio.

For the prospective study, patients were pretreated with 300 mg potassium perchlorate orally 30 min before administration of [^{99m}Tc]HM-PAO.

In a comparative study, 19 patients received 111 MBq (3 mCi) [^{123}I]IMP and image quality was assessed as described above.

The statistical significance of differences between two groups was assessed with the t-test and between more than two groups with the F-test (*9*).

RESULTS

Table 1 presents initial radiochemical purity (RCP) as a function of the manufacturer's batch of kits. RCP did not vary significantly between batches (F-test, $p > 0.1$). In addition, there was no trend toward lower RCP in the more recent batches despite the addition of larger amounts of pertechnetate to these kits. On only four occasions was RCP <80%, the limit of acceptability set by the manufacturer (*4*); on these occasions the quantities of pertechnetate added were 1200, 1256, 1965, and 2100 MBq.

TABLE 1
Batch-to-Batch Variation in Initial Radiochemical Purity (RCP) of [^{99m}Tc]HM-PAO

Batch	N	MBq added	% RCP	Range
A19	4	1573 ± 342	86.0 ± 2.0	(84.6–88.7)
A24	4	1514 ± 285	85.0 ± 5.2	(79.5–89.8)
A26	4	1623 ± 349	88.5 ± 4.7	(82.0–92.0)
A29	12	1620 ± 475	86.5 ± 4.9	(75.0–91.5)
A36	20	1691 ± 295	87.3 ± 3.6	(80.0–92.7)
A43	32	2220 ± 453	86.9 ± 3.6	(77.0–91.2)
A47	10	2266 ± 540	89.5 ± 1.8	(87.6–92.4)
A53	13	1399 ± 454	87.9 ± 2.7	(82.3–91.8)

Each value is mean ± s.d. for N determinations.

TABLE 2
Effect of Generator In-Growth and Time Since Elution of Pertechnetate on Initial RCP of [^{99m}Tc]HM-PAO

Parameter	N	MBq added	% RCP
Generator in-growth (hr)			
1–6	19	1965 ± 415	88.0 ± 4.0
24	24	2378 ± 522	89.0 ± 2.8
Hr since elution			
<1	42	1892 ± 476	86.9 ± 3.5
1–2	14	2263 ± 414	88.4 ± 3.7
2–3	12	1770 ± 383	87.4 ± 3.3
3–4	7	2095 ± 707	85.8 ± 3.3

Each value is mean ± s.d. for N determinations.

Table 2 presents initial RCP as a function of generator in-growth and time since elution of the generator. There was no difference between 1–6 and 24 hr of generator in-growth. Similarly, the time since elution of the generator, varying from <1 to 4 hr, did not affect RCP. Pertechnetate eluted >4 hr prior to reconstitution time was not used.

Table 3 presents the batch-to-batch variability in image quality as reflected by the count densities in the parotid glands and nasal region as ratios to the basal structures. The placement of the regions of interest is shown in Figure 1. Due to the variability within batches, the differences between batches were not significant (F-test, $p > 0.1$).

Table 4 presents the effect of the amount of activity added to the kit on the resultant image quality. When <3000 MBq pertechnetate was added, initial RCP and parotid/basal ratios were not significantly affected by the amount of activity. Indeed, addition of <1500 MBq or 2501–3000 MBq resulted in identical RCP and similar parotid ratios. However, when >3000 MBq was added, RCP tended to be lower (F-test, $p \sim 0.1$) and parotid/basal ratios were significantly higher (F-test, $p < 0.05$).

The effect of injection delay (min post reconstitution) on image quality is presented in Table 5. For this table, the RCP at time of injection is reported, either determined by measurement at that time or estimated from

TABLE 3
Batch-to-Batch Variability in Image Quality

Batch	N	% RCP	Ratio to basal structures: Parotid	Ratio to basal structures: Nasal
A	7	86.4 ± 4.8	0.74 ± 0.09	0.44 ± 0.10
B	8	88.9 ± 1.4	0.75 ± 0.10	0.43 ± 0.07
C	24	86.3 ± 4.0	0.74 ± 0.12	0.42 ± 0.09
D	8	90.9 ± 2.3	0.70 ± 0.11	0.39 ± 0.12
E	6	88.6 ± 2.8	0.81 ± 0.11	0.45 ± 0.11

Each value is mean ± s.d. for N determinations.

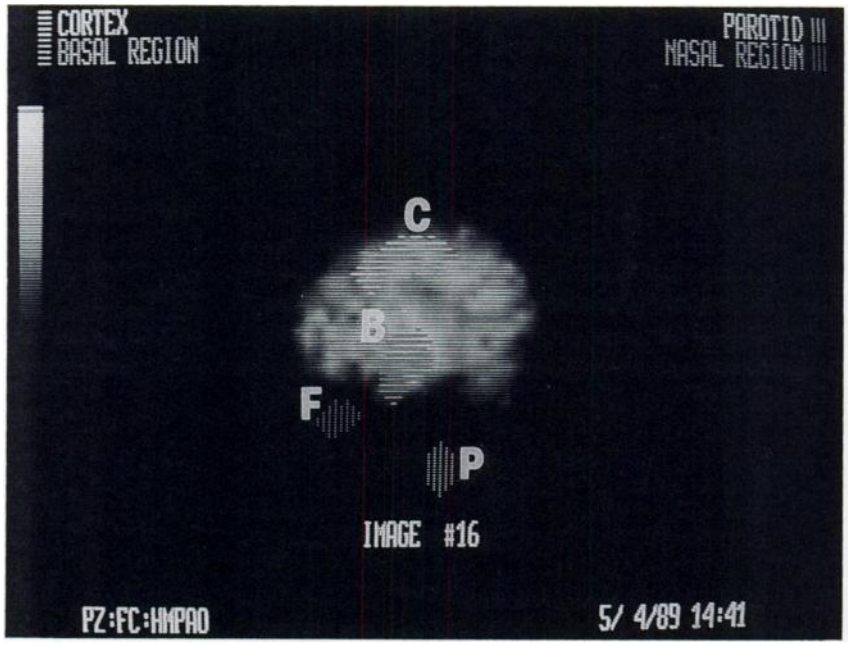

FIGURE 1
Left lateral projection image showing the placement of regions of interest over the cerebral cortex (C), temporal lobe and basal ganglia (B), facial area (F), and parotid gland (P).

the initial RCP and decomposition rate of 0.17 hr^{-1} reported previously (*7,8*) and reproduced in our laboratory. Within 20 min of reconstitution, RCP averaged >85% and parotid/basal ratios remained low, whereas the lower RCP obtained >20 min after reconstitution resulted in sharply higher parotid/basal ratios. The differences in RCP and parotid ratios were statistically significant (F-test, $p < 0.005$ and $p < 0.025$, respectively) and there was a strong correlation between average RCP and average parotid ratio ($r = -0.99$, $p < 0.01$).

In an attempt to improve image quality by blocking parotid uptake of free pertechnetate, 30 patients were pretreated with 300 mg potassium perchlorate. As shown in Table 6, this maneuver significantly reduced the parotid/basal ratios (t-test, $p < 0.001$) without affecting the nasal ratios. However, the parotid ratios remained higher than those obtained with [^{123}I]IMP (t-test, $p < 0.025$).

The data in Table 7 show that RCP at the time of injection directly affected image quality ($r = -0.40$, $N = 61$, $p < 0.01$; F-test, $p < 0.025$) but pretreatment with perchlorate eliminated this effect ($r = -0.01$, $N = 27$, $p > 0.1$; F-test, $p > 0.1$).

Finally, Figure 2 presents coronal views of two patients who received [^{99m}Tc]HM-PAO from the same vial. During injection of the first dose, blood was accidentally drawn into the syringe and allowed to incubate before injection, resulting in poor image quality (Fig. 2A). The second dose from the same vial was injected cleanly 13 min later and image quality was good (Fig. 2B). Similarly, interstitial injection resulted in poor image quality; there was a substantial amount of extracranial activity, particularly in the parotid glands.

DISCUSSION

These data show that the radiochemical purity (RCP) of [^{99m}Tc]HM-PAO varied between preparations but that the variability within batches was as great as that between batches (Table 1). The manufacturer recommends that the pertechnetate be used within 4 hr from a generator eluted <24 hr previously (*4*). Within these limits, the length of in-growth and time since elution

TABLE 4
Effect of Amount of Activity Added to Kit on Image Quality

MBq added	N	% RCP	Parotid/ basal
<1500	4	89.0 ± 2.1	0.71 ± 0.06
1501–2000	17	85.5 ± 4.3	0.72 ± 0.13
2001–2500	18	87.8 ± 3.3	0.82 ± 0.14
2501–3000	10	89.0 ± 2.3	0.74 ± 0.09
>3000	5	84.1 ± 6.1	1.10 ± 0.28

Each value is mean ± s.d. for N determinations.

TABLE 5
Effect of Injection Delay on Image Quality

Minutes post-recon	N	% RCP*	Parotid/ basal
≤10	20	87.7 ± 3.4	0.73 ± 0.11
11–20	21	86.1 ± 4.1	0.75 ± 0.13
21–30	10	81.1 ± 4.3	0.89 ± 0.25
≥31	6	78.8 ± 5.3	0.91 ± 0.21

Each value is mean ± s.d. for N determinations.
* Estimated % RCP at time of injection.

TABLE 6
Comparison of Image Quality Between [^{99m}Tc]HM-PAO With and Without Perchlorate Pretreatment and [^{123}I]IMP

Radiopharmaceutical	N	Ratio to basal structures: Parotid	Nasal
HM-PAO, control	25	0.74 ± 0.11	0.42 ± 0.09
HM-PAO, perchlorate	30	0.63 ± 0.11	0.37 ± 0.10
IMP	19	0.56 ± 0.08	0.38 ± 0.06

Each value is mean ± s.d. for N determinations.

TABLE 7
Effect of Radiochemical Purity at Time of Injection on Image Quality With and Without Perchlorate Pretreatment

	Control		Perchlorate	
% RCP*	N	Parotid/basal	N	Parotid/basal
≤80	11	0.89 ± 0.19	1	0.54
81–85	13	0.82 ± 0.24	3	0.60 ± 0.12
86–90	31	0.73 ± 0.10	14	0.66 ± 0.12
≥91	6	0.67 ± 0.09	9	0.59 ± 0.10

Each value is mean ± s.d. for N determinations.
* Estimated % RCP at time of injection.

did not significantly affect RCP (Table 2). Anecdotal evidence suggests that exceeding these limits can adversely affect initial RCP and/or stability.

Similar to RCP, image quality as reflected by parotid uptake, varied within batches as much as between batches (Table 3).

The manufacturer recommends that a maximum of 1110 MBq (30 mCi) pertechnetate be added to the kit (*4*). This is apparently based on the data presented by Neirinckx (*1*). In single preparations, Neirinckx (*1*) and Jia (*10*) showed that addition of 9400–9470 MBq (254–256 mCi) pertechnetate resulted in poor stability; analysis of the data presented in those two papers produces a k_d value of ~0.5 hr^{-1}, triple the usual rate constant of 0.17 hr^{-1} (*7,8*, and reproduced in our laboratory). However, analysis of data following addition of 3267 MBq (88.3 mCi) and 5550 MBq (150 mCi) pertechnetate by Hung (*8*) and Jia (*10*), respectively, produced normal k_d values of ~0.17 hr^{-1}. Our results (Table 4) show that 3000 MBq (81 mCi) pertechnetate could be added to the kit without adversely affecting image quality; this is almost triple the amount recommended by the manufacturer and allows injection of up to four doses per vial. However, the "quality" of the pertechnetate must be good and doses must be injected soon after reconstitution. Table 5 shows that image quality sharply declined when RCP was <85%, which occurred ~20 min after reconstitution.

We prospectively studied a group of patients who were pretreated with 300 mg potassium perchlorate 30 min before injection of [^{99m}Tc]HM-PAO. This pretreatment significantly reduced the parotid/basal ratios compared to patients who were not pretreated but received [^{99m}Tc]HM-PAO from the same batches (Table 6). The nasal ratios were not affected by perchlorate. Presumably perchlorate would also block uptake of pertechnetate by the choroid plexus which could affect scan

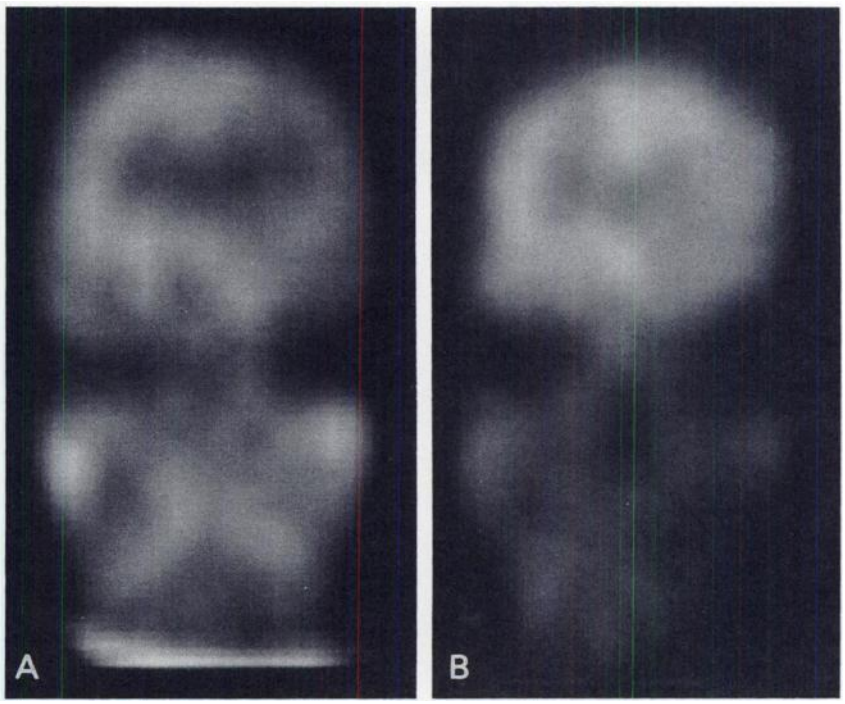

FIGURE 2
These two patients received injections of [^{99m}Tc]HM-PAO from the same vial. Coronal images were reconstructed at the plane of the parotid glands including the face and neck to demonstrate the degree of extracranial activity. A: Patient who received first injection. Blood was accidentally drawn into the syringe before injection. Extensive extracranial activity is noted. B: Patient who received second injection from same vial, 13 min later. The injection was performed cleanly. Image quality is good.

interpretation. However, free pertechnetate was not the only species affecting image quality; parotid ratios in the perchlorate-treated patients were still higher than those observed with [^{123}I]IMP (Table 6).

We believe that the data in Table 7 show that RCP of [^{99m}Tc]HM-PAO directly affects image quality. The manufacturer states that the minimum acceptable RCP is 80% (*4*). However, the publication on which that recommendation is based states that "in all cases, the radiopharmaceutical was used with proportion of the primary ^{99m}Tc complex never <85%" (*1*). Similar criteria were used by Sharp (*11*). Our data (Table 7) show that image quality drops sharply when RCP falls below 85% and we feel that 85% is a more appropriate limit, although pretreatment with perchlorate may allow this to be relaxed (Table 7).

However, RCP is not the only factor which affects image quality. Injection technique is also important. Allowing blood to mix with [^{99m}Tc]HM-PAO (Fig. 2) and interstitial injection can result in poor image quality. Ell has previously shown in rats that incubation of [^{99m}Tc]HM-PAO with blood before injection results in lower brain uptake (*12*).

Based on this review of our experience with [^{99m}Tc] HM-PAO imaging, we recommend the following.

1. Patients should be pretreated with potassium perchlorate to block uptake of free pertechnetate.

2. RCP of [^{99m}Tc]HM-PAO should be checked immediately before each injection and should be ≥85%. The rapid extraction technique makes this practical (*6*).

3. [^{99m}Tc]HM-PAO should be injected cleanly through a butterfly to prevent mixing with blood in the syringe.

It has been shown that [^{99m}Tc]HM-PAO can be stabilized in vitro by adjustment of pH and addition of an antioxidant (*8*), but there is not yet a simple and reproducible method to accomplish this. Our data underscore the importance of maintaining high RCP of [^{99m}Tc]HM-PAO.

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