Influence of pH Adjustment Agents on the Biologic Behavior of Osmium-191 Impurity in Irridium-191m Generator Eluates

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The influence of four pH adjustment agents on the biologic behavior of osmium-191 (^{191}Os) impurity in ^{191}Os/^{191m}Ir generator eluates was studied. Extended body clearance and biodistribution studies were performed in mice. The solutions to be injected were obtained by eluting generators with a 0.9% NaCl solution at pH 1. The pH of these eluates was adjusted to 5–9 with succinate, phosphate, lysine or NaOH solution. Our results demonstrate that the biologic behavior of these generator eluates is significantly dependent on the agent used for pH adjustment. Buffering with lysine leads to the best results: (a) the mice show no adverse reaction after injection of 150 human doses and the body clearance is very rapid and (b) more than 75% I.D. at 24 hr postinjection. Preliminary calculations based on these results suggest a significant decrease in the estimated patient radiation dose when lysine buffered ^{191}Os/^{191m}Ir generator eluates are used for radionuclide angiography.

J Nucl Med 1990; 31:000–000

Iridium-191m (^{191m}Ir), an ultrashort-lived radionuclide ($T_{½}$ = 4.96 sec), has been proven to be suitable for diagnostic imaging with conventional gamma cameras (*1,2,3,4*). This radioisotope can be recovered from its parent osmium-191 (^{191}Os) ($T_{½}$ = 15.4 d) by using suitable separation systems. Recently developed high-performance generators (*5,6,7,8*) yield ^{191m}Ir in amounts adequate for high quality radioangiography studies, accompanied by very low ^{191}Os breakthrough. Generally, ^{191m}Ir is eluted from the generators by acidic saline solution and is thereafter adjusted as closely as possible to physiologic pH before injection (*5,6,8*). The main advantage of the clinical use of ^{191m}Ir is that it makes it possible to perform multiple angiograms at short time intervals without any background interference and with very low radiation dose to the patient.

Received July 30, 1989; revision accepted Nov. 14, 1989.

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We recently developed an improved ^{191}Os/^{191m}Ir generator system, which has proven to be suitable for first-pass radionuclide angiography (*4,8*). During its 3-wk shelf-life the yield in a 2-ml eluate goes from 250 to 100 mCi ^{191m}Ir, accompanied by 5-3 μCi ^{191}Os breakthrough. Before in vivo injection, the pH of the acidic eluate (saline pH 1) is adjusted with succinate buffer. Due to its very short half-life, the contribution of ^{191m}Ir to the radiation absorbed by the patient is very low: 1.2 mrad/100 mCi (*6*). The radiation doses are mainly due to the traces of the ^{191}Os present as a contaminant in the generator eluate. The wholebody radiation dose we calculated is 0.6 mrad/μCi ^{191}Os using succinate for buffering of the eluate. The highest single-organ dose we found is 7.8 mrad/μCi to the liver (*8*).

In an effort to extend the use of ^{191m}Ir to continuous infusion, several patients were given a 1-min injection of 20 ml succinate-buffered eluate. In certain cases, this led to moderate pain along the injected vein and a metallic taste in the mouth which caused slight coughing. One patient developed facial flushing during the injection. However, all such symptoms of discomfort disappeared spontaneously within a few minutes.

Because we were anxious to avoid all adverse reactions while maintaining the ability of the generator to provide high specific activity ^{191m}Ir in the buffered eluate, four pH adjustment agents were tested and the biologic behavior of these eluates was studied in mice. We demonstrated that the behavior of the ^{191}Os impurity in ^{191}Os/^{191m}Ir generator eluate is different with each pH adjustment agent.

MATERIALS AND METHODS

^{191}Os/^{191m}Ir Generator

The ^{191}Os/^{191m}Ir generator system that we developed has already been described (*7*). The system is composed of two columns in series: a main generator column, packed with silica gel impregnated with tridodecylmethyl-ammonium chloride and loaded with high activity ^{191}Os as osmyl chloride, and a scavenger column packed with activated charcoal. Irridium-191m is eluted from the generator by pH 1 saline. This

system, which is loaded with ~1 Ci ^{191}Os, is characterized by a high elution yield (~25% ^{191m}Ir recovery with ~ 5×10^{-4}% ^{191}Os breakthrough) and a shelf-life of more than 3 wk. The eluate contains no ^{192}Ir, as it is removed in an early stage of the complex preparation, before the osmium is loaded onto the generator column (*8,9*). The buffered eluate is sterile, pyrogen-free, and nontoxic.

Concentration and volume of the pH adjustment agents were added to 3 ml of generator eluate: 0.8 ml of 1.20 *M* Succinic acid disodium salt hexahydrate solution, (Riedel-de-Haen), 0.45 ml of 0.5 *M* Di-sodium hydrogen orthophosphate dodecahydrate, (BDH - Analar), 0.2 ml of 1 *M* L-lysine monohydrocholoride, (Aldrich), and 0.8 ml of 0.24 *M* NaOH, (Frutarom).

Eluates

The present study was performed on generators from two batches. The generators were washed with 30 ml pH 1 saline before the solutions to be used for in vivo testing were eluted. Particularly small scavenger columns were used in order to obtain as much as 10–40 μCi ^{191}Os/ml in the 3-ml eluates. These activity levels permitted acceptable counting statistics throughout the study.

In all of our experiments, fresh elutions were used and their pH was adjusted as closely as possible to physiologic values, using one of the agents listed in Table 1.

Biologic Studies

Four groups of 12–18 ICR mice weighing 19–25 g were studied. pH-Adjusted generator eluate (0.2 ml) containing at least 1 μCi of ^{191}Os was injected into the tail vein of each mouse. Whole-body radioactivity retention and biodistribution studies were performed in all the mice. The measurements were performed in a gamma counting chamber. After injection, each mouse was placed in an aerated container and was counted for a determination of the injected dose. Whole-body retention was measured and calculated as percent of the injected dose (%I.D.). Counting started 2–3 hr postinjection and continued at different time intervals either for 4 wk or until body retention was <10% I.D. Every time retention was measured, two to four animals from each group were killed and biodistribution was determined by counting the ^{191}Os activity of six organs and of samples of blood, muscle, bone, urine, and feces. The values were calculated as %I.D.

TABLE 1
Concentration and Volume of pH Adjustment Agents and Final pH of the Injected Materials

	Concentration (*M*)	Volume (ml)*	pH
Succinate†	1.20	0.80	4.5
Phosphate‡	0.50	0.45	5.1
Lysine§	1.00	0.20	8.7
NaOH¶	0.24	0.80	4.5

* Added to 3 ml generator eluate.
† Succinic acid disodium salt hexahydrate.
‡ Di-sodium hydrogen orthophosphate dodecahydrate.
§ L-lysine monohydrochloride.
¶ Frutarom.

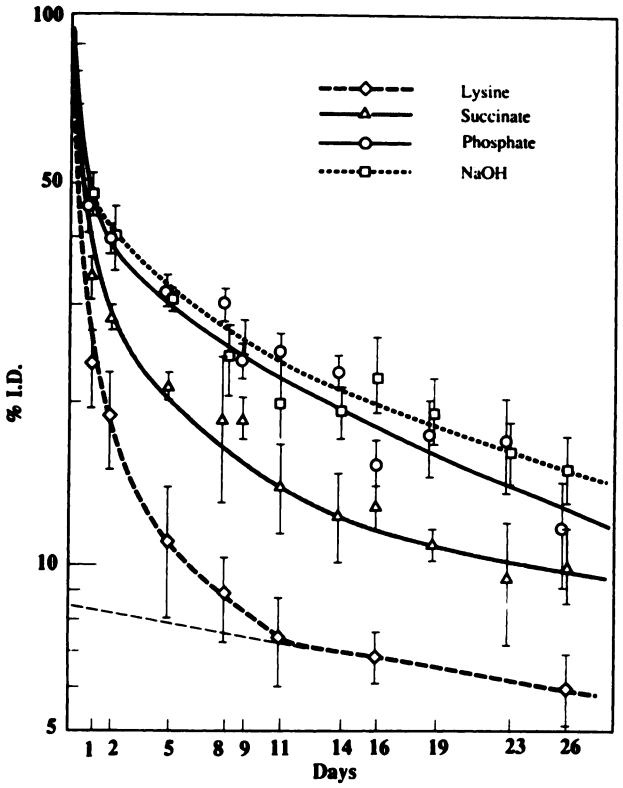

FIGURE 1
Body retention of ^{191}Os/^{191m}Ir generator eluates after pH adjustment with succinate, phosphate, lysine, and NaOH solutions.

RESULTS AND DISCUSSION

The aim of the present study was to evaluate the influence on the biologic behavior in mice of ^{191}Os-impurity in ^{191}Os/^{191m}Ir generator eluate when four neutralizing agents (succinate, phosphate, lysine and NaOH) were used for pH adjustment. Slight adverse reactions were noted when mice were injected with ~100 human doses (HD), calculated on the basis of a dose/kg-body weight equivalent, using succinate, phosphate and NaOH pH-adjusted eluates. No reaction was seen after injection of 150 HD of lysine buffered eluate.

The body retention of mice injected with lysine, succinate, phosphate and NaOH pH-adjusted generator eluate is shown in Figure 1. Buffering with lysine resulted in the quickest body clearance: <10% I.D. was

TABLE 2
Body Retention of ^{191}Os/^{191m}Ir Generator Eluates After pH Adjustment with Phosphate, NaOH, Succinate, and Lysine

Buffer	Days after injection	Body retention (% I.D.)
Phosphate	23	16.9 ± 3.2
NaOH	23	16.1 ± 2.0
Succinate	23	9.4 ± 1.2
Lysine	11	7.3 ± 1.3

TABLE 3
Biodistribution (% I.D.) of $^{191}Os/^{191m}Ir$ Generator Eluates After pH Adjustment with Phosphate, NaOH, Succinate, and Lysine

Organ	Phosphate*	NaOH*	Succinate*	Lysine†
Blood	8.10 ± 0.07	0.57 ± 0.05	0.65 ± 0.30	0.07 ± 0.01
Bones	1.60 ± 0.05	5.93 ± 0.50	3.10 ± 0.15	0.70 ± 0.04
Muscles	1.40 ± 0.50	4.10 ± 1.30	2.42 ± 0.07	2.38 ± 0.26
Spleen	2.40 ± 0.40	0.38 ± 0.10	0.42 ± 0.12	0.16 ± 0.05
Kidneys	0.30 ± 0.50	0.45 ± 0.10	0.21 ± 0.02	1.00 ± 0.50
Stomach	0.07 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.95 ± 0.05
Gut	0.72 ± 0.04	1.00 ± 0.15	0.63 ± 0.03	0.94 ± 0.02
Liver	2.11 ± 0.01	3.25 ± 0.60	1.70 ± 0.20	1.50 ± 0.30
Lungs	0.19 ± 0.01	0.16 ± 0.01	0.16 ± 0.04	0.17 ± 0.25

* 23 days after i.v. injection.
† 11 days after i.v. injection.

retained a week after injection. The retained dose was double for succinate and about three times higher for phosphate and NaOH adjustment throughout the whole experiment (Table 2).

The biodistribution for the previously mentioned body retentions was calculated and is shown in Table 3. In this table, the values for succinate, phosphate and NaOH are shown at 23 days postinjection; whereas for lysine they are shown at 11 days, because of its very fast body clearance. The biologic behavior was almost identical for NaOH and succinate groups, for which ~30% of the retained dose (R.D.) was found in the bones, 30% in the muscles, and ~20% in the liver (Table 2). For the phosphate group, the highest uptake was in the blood—almost 50% R.D., ~10% in the bones and muscles, respectively, and 12% in the liver. Of the very low body retention of the lysine group, 38% R.D. was distributed in the muscles, i.e. in 45% of the body weight, and 20% in the liver. This means that most of the small amount of activity retained in the mice of the lysine group was distributed over almost half of the body weight and was not retained in significant quantities by the blood, bones, or liver.

The body-retention curve for these mice, together with the corresponding biodistribution values, suggest that their absorbed radiation doses were lower than those calculated for mice given the succinate-buffered eluate (*8*). On the basis of these results, preliminary calculations indicate a significant decrease in the estimated patient radiation dose when lysine-buffered $^{191}Os/^{191m}Ir$ generator eluates are used for radionuclide angiography.

The present results demonstrate that the biologic behavior of the ^{191}Os breakthrough in the generator eluate is significantly dependent on the agent used for its pH adjustment. It seems reasonable to assume that this dependence is a result of different interactions between osmium species and various functional groups present in the agents used for pH adjustment. Further investigations are necessary to improve understanding of these interactions.

CONCLUSION

The present work demonstrates that in mice body retention and biodistribution of ^{191}Os-impurity in the acidic eluate of $^{191}Os/^{191m}Ir$ generators are affected by the agent used for pH adjustment. Of the four agents studied, lysine leads to the shortest biologic half-life. The mice showed no reaction after the injection of more than 150 HD buffered-generator eluate. Its rapid body clearance, as well as its biodistribution pattern, make lysine-buffered $^{191}Os/^{191m}Ir$ generator eluates suitable for radionuclide angiography in young children, for whom very low radiation doses are essential.

ACKNOWLEDGMENTS

The authors thank Janet Shalev, Nurit Kanyon, and Leah Nechemia for the excellent technical assistance. They also thank Mrs. Hilda Krummbein for her help in preparing this manuscript.

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