Radiochemical Purity and In Vitro Stability of Commercial Hippurans

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Good radiochemical purity of hippuran is important regarding the patient's radiation dose, primarily because of the high thyroid exposure from free iodide. The radiochemical purity and in vitro stability of 11 commercially available ^{131}I, ^{125}I, and ^{123}I hippurans were analyzed by means of thin layer chromatography. Three different radioactive impurities were found in all hippuran samples: (a) free radioiodide, (b) a radiochemical impurity that has been unknown up until now, and (c) labeled *o*-iodobenzoic acid. Quality control of several hippurans with regard to radiochemical purity gave evidence of considerable differences during storage. The lowest amount of free iodide was 0–0.6% in a hippuran preparation from one manufacturer, the highest amount 0.8–4.6% in a sample from another manufacturer. Hippuran samples from four other manufacturers were found to contain > 2% of free iodide prior to their expiration date.

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One of the most frequently used radiopharmaceuticals in nuclear medicine is *o*-iodohippuric acid labeled with radioactive iodine (hippuran). Iodine-131 (^{131}I), iodine-125 (^{125}I), and iodine-123 (^{123}I) hippurans are used for various kidney examinations. Good radiochemical purity of hippuran is important regarding the patient's radiation dose, especially in view of the high thyroid exposure from free iodide (*1*).

The purpose of this study was to test the radiochemical purity of ^{131}I, ^{125}I, and ^{123}I hippurans available commercially, i.e., the measure of the percentage of radiochemical impurities including their chemical identity in relation to the total radioactivity of the pharmaceutical. Also tested was the in vitro stability of the drugs.

Three different radiochemical impurities were found in all hippuran samples: (a) radioiodide, (b) a radiochemical impurity that has been unknown up until now, and (c) a labeled *o*-iodobenzoic acid. There are two reasons for the radioiodide impurity: (a) residues from manufacturing, since *o*-iodohippuric acid is labeled with radioiodine by isotope exchange; and (b) decay products due to the in vitro instability. Labeled *o*-iodobenzoic acid is due to an impurity of the initial material *o*-iodohippuric acid.

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MATERIALS AND METHODS

The samples selected for quality control with regard to radiochemical purity and in vitro stability were taken from six different manufacturers or distributors of [^{131}I]hippuran, two manufacturers of [^{125}I]hippuran and three manufacturers of [^{123}I]hippuran.

We examined 11 batches of [^{131}I]hippuran, two batches of [^{125}I]hippuran and six batches of [^{123}I]hippuran. The radiopharmaceuticals selected for analysis, the different manufacturers, and the number of batches examined are listed in Table 1. From nearly all manufacturers, two batches were analyzed and examinations were performed for each batch at different times to study decomposition of $^{131}I/^{125}I/^{123}I$ hippurans, from time of receipt to the expiration date, in general using for each time four parallel chromatograms.

To study the decomposition of [^{123}I]hippurans with a half-life of 13.2 hr of the radioisotope, ^{123}I products were additionally tested 1 day after the expiration date. All hippurans were stored under the conditions specified in the operational instructions in a refrigerator and protected from light.

Chromatographic techniques were used to assess the radiochemical purity. Iodine-131, ^{125}I, and ^{123}I hippurans were analyzed by means of ascendant thin layer chromatography (TLC) using TLC plastic sheets (20 cm × 20 cm) silica gel F 1500 LS 254* with mobile phase chloroform/glacial acetic acid 90:10 (*2,3*). In order to prevent loss of carrier-free iodide during application of the samples (10 μl) and subsequent chromatography, a carrier solution was added to the spotting solution (5:1). The carrier solution was prepared as follows. Forty milligrams *o*-iodohippuric acid and 40 mg *o*-iodobenzoic acid were dissolved in 4 ml 0.1 N NaOH, 10 mg potassium iodide were added, and the mixture was filled up with water

TABLE 1
Examined Hippurans and Their Manufacturers/ Distributors

Internal product designation	Hippuran	Manufacturer/ distributor	Number of batches
A	^{131}I	EIR/Squibb	3
C	^{131}I	Hoechst	2
D	^{131}I	Amersham	2
E	^{131}I	Amersham/Mallinckrodt	2
F	^{131}I	Sorin/CIS	1
G	^{131}I	ZfK/IRE	1
H	^{125}I	Hoechst	1
K	^{125}I	Amersham	1
L	^{123}I	EIR/Squibb	2
M	^{123}I	Amersham	2
N	^{123}I	IRE	2

to a total volume of 10 ml. This solution was stored in a refrigerator. The chromatogram was developed at room temperature during 1¾ hr after which time the front had proceeded ~17 cm. After drying, counting (10 min) was performed with a position sensitive gas flow counter (TLC-Linear Analyzer LB 283*). This instrument gives information about the position of beta- and gamma-ray sources in addition to detecting ionizing radiation and counting the whole chromatogram trace simultaneously (*4–7*).

When performing TLC by the above-mentioned separation method, the problem arises that everything remaining at the start of chromatography is interpreted as iodide. However, other impurities, for example decomposition products, could also be involved.

To clarify the fact that the activity remaining at the start is produced solely by iodide, the following experiment was made. The radiopharmaceutical (^{131}I *o*-iodohippuric acid) was chromatographed by the previously used separation method and the activities were measured by a TLC linear analyzer. The thin-layer plate was then turned by 90° and chromatographed in the new mobile phase (80% methanol) (*8*) (development time 50 min; flow distance 9 cm). The activities were measured again.

RESULTS

The separation technique is sensitive to the following radioactive substances: free iodide, hippuran, *o*-iodobenzoic acid, glycyl hippuran, and an unknown impurity. The iodide impurity remains at the origin (Rf=0), and for the radiopharmaceutical an Rf value of ~0.4 is determined. An unknown radiochemical impurity reaches an Rf value of 0.5 to 0.7, and *o*-iodobenzoic acid is nearby the solvent front (Rf=0.8 to 0.9). Another radiochemical impurity with an Rf value of 0.25 is considered to be glycyl hippuran according to a previous study (*9*). This impurity could be detected only in some products.

The amount of radiochemical impurities (free iodide, unknown impurity, and *o*-iodobenzoic acid) consists of the initial amount at the time of manufacturing and the amount induced by decomposition. With the chromatographic method described, decomposition was observed in all hippurans from times of receipt to the expirations dates. For example, in product F ([^{131}I] hippuran), the percent values of free radioiodide, unknown radiochemical impurity, and labeled *o*-iodobenzoic acid increase over a time period of 34 days (Table 2) (four radiochromatograms for each time; mean value ± 1 s.d.). In all the other [^{131}I]hippuran products an increase in the radiochemical impurities could also be observed. If the evaluated stability of [^{123}I] hippuran is taken into account, it may be concluded from this study that the percentage of iodide, unknown impurity, and *o*-iodobenzoic acid increases also during storage (24 hr). The two ^{125}I hippurans also showed an increase in the radiochemical impurities during storage (1 mo).

A survey of all analyses (radiochemical purity and in vitro stability of hippurans as a function of time) is given in Table 3, where the percent values of the radiopharmaceutical and the impurities are shown as determined in each individual batch. First numbers indicate the minimum amount found in hippuran(s) after receipt (first measurement), and second numbers indicate the maximum amount previous to the expiration date (last measurement).

DISCUSSION

Many methods of determining the radiochemical purity of hippuran have been described in the literature (*8*). Thin layer chromatography used in the present study has a decisive advantage in comparison to other separation methods. It enables the following four radiochemical impurities to be identified in addition to hippuran: radioiodide, glycyl hippuran, an unknown radiochemical impurity, and *o*-iodobenzoic acid. Most of the separation methods that have been used until now only enabled iodide and *o*-iodobenzoic acid to be identified in addition to hippuran. The unknown impurity has also been noted in other reports (*9–11*). This impurity is likely an organic compound, e.g., labeled 2,4,6 triiodohippuran (*12,13*).

To prevent possible loss of radioiodide and iodine-labeled compounds during the application of the sample and subsequent chromatography by volatilization, a carrier solution was mixed to the spotting solution. This

TABLE 2
In Vitro Stability of [^{131}I]Hippuran (F)

Days after receipt	% Iodide	% Hippuran	% o-Iodo-benzoic acid	% Unknown Impurity
+6	0.38 ± 0.02	98.48 ± 0.08	0.39 ± 0.05	0.75 ± 0.04
+26	1.15 ± 0.04	97.47 ± 0.05	0.52 ± 0.04	0.83 ± 0.03
+40	2.76 ± 0.22	95.27 ± 0.05	0.70 ± 0.06	1.16 ± 0.07

TABLE 3
Radiochemical Purity of $^{131}I/^{125}I/^{123}I$ Hippurans as Function of Time

Hippuran	Batch	Time period (days)	% Iodide	% Hippuran	% o-Iodo-bezoic acid	% Unknown impurity
A	1	7	1.32–2.51	96.41–97.59	0.76–0.77	0.15–0.30
	2	14	0.75–1.37	97.98–98.58	0.49–0.79	0.03–0.20
	3	13	1.49–4.62	93.74–97.80	0.58–1.24	0.14–0.37
C	1	14	0.28–0.71	99.03–99.51	0.02	0.21–0.24
	2	3	0.84–0.91	98.83–98.93	0–0.01	0.23–0.25
D	1	14	0.07–0.64	99.18–99.85	0.06	0.03–0.12
	2	3	0.53–0.58	99.28–99.30	0.03–0.05	0.11–0.12
E	1	14	0.73–0.74	98.92–99.09	0.04–0.09	0.14–0.27
	2	10	0.69–2.70	96.37–98.99	0.08–0.20	0.24–0.61
F	1	34	0.38–2.76	95.27–98.48	0.39–0.70	0.75–1.16
G	1	14	0.46–2.08	96.98–98.90	0.14–0.22	0.51–0.72
H	1	20	1.33–1.71	96.80–97.68	0.34–0.51	0.14–0.37
K	1	26	0–0.81	98.75–99.58	0.13–0.21	0.17–0.25
L	1	0.5	1.30	97.65	1.09	0
	2	1	1.19–1.95	97.43–97.93	0.47–0.62	0–0.03
M	1	1	0.04–0.16	99.19–99.62	0.22–0.27	0
	2	1	0.69–1.66	98.14–98.94	0.15–0.22	0.06–0.15
N	1	0.5	1.93–2.23	97.51	0.42	0.13
	2	1	1.76–2.39	97.14–97.62	0.31–0.43	0.17–0.20

is a well-known technique for all iodine-labeled compounds.

The only disadvantage of this separation procedure is the time needed. The mobile phase needs about 1¾ hr for 18 cm. This time factor plays no role in the case of ^{131}I and ^{125}I products with a half-life of 8.04 or 60.0 days, but for ^{123}I with a half-life of 13.2 hr, a faster separation procedure must be found since too much time is needed for quality control before delivery of the product and before use in man. In an experiment, it was possible to find out whether the free iodide separated by means of thin layer chromatography would be radiochemically contaminated by iodate. Since the second mobile phase (80% methanol) specifically separates iodide from iodate and the proportional activity at the start of the first separation (iodide + possibly iodate) is in agreement with the proportional activity at the front (iodide) of the second separation, it may be assumed that iodide is not contaminated by iodate and that no further radiochemical impurities are contained in the proportional amount of iodide activity.

When the stability of a radiopharmaceutical is considered, the effects of ionizing radiation (physical half-life, type, and energy of radiation) in the preparation have to be taken into account in addition to hydrolysis and oxidation. As indicated in the literature (*14,15*), the decomposition of [^{131}I]hippuran may be attributed to the radiation absorbed in most cases. For example, ^{131}I in the form of free iodide can be split from a [^{131}I] hippuran. This is due to radiolysis of water and the reaction of products with the labeled hippuran. As far as the energy of ionizing radiation is absorbed in a diluted solution, this is primarily due to interaction with the molecules of the solvent that are excited or decomposited during this process.

A series of publications shows how radiochemical impurities of otherwise faultless preparations may lead to inaccurate diagnoses. An amount of over 2% of free radioactive iodide in hippuran may thus be the cause of errors occurring in clearance tests (*16*). Amounts of free radioiodide result in an undue radiation exposure of the thyroid. Consequently, 3% of free radioiodide from a typically administrated activity of 30 μCi (1.11 MBq) [^{131}I]hippuran will increase the energy dose to ovaries by 62%, to testes by 68%, and to red bone marrow by 131% compared with the pure radiopharmaceutical; the value of the energy dose to the thyroid, as influenced by the free iodide, amounts to 1,890 mrad (18.9 mGy) (*1*). The contribution to the clearance is unknown for both glycyl hippuran and *o*-iodobenzoic acid, the latter only being described in liver function studies (*9*). This is why there is a demand for preparations to be as pure as possible, as described in respective monographies of the pharmacopias (*17,18*). According to the requirement dictated in the European Pharmacopoeia (EP) (*17*) the following limits of radiochemical purity have to be observed in the case of [^{131}I]hippuran: hippuran ≧95%, max 3% iodide, max 2% *o*-iodobenzoic acid. The United States Pharmacopoeia (USP) (*18*) requires that the radioactivity under the hippuran band of a chromatogram is not <97% of the total radioactivity. USP XXI as well as EP contain no monographies on $^{125}I/^{123}I$ hippurans.

Radiochemical purity depends also upon the stability of hippuran as a function of time, temperature, pH, exposure to light, and internal irradiation. Our examinations adhered to the storage conditions prescribed by the manufacturers where temperature (refrigerator) and exposure to light (dark) are concerned. The pH range specified by the manufacturers was not considered in

our tests. As shown in Table 3, the amount of radiochemical impurity as a function of storage time increases. In the examined ^{131}I hippurans (A-G), large differences can be seen between the amounts of free iodide after receipt and shortly before expiration. A high content of free iodide after receipt is the result of inadequate purification after labeling, while high percentages of iodide shortly before expiration are indicative of a low degree of stability as a function of time. For example, in Product F the percentage of free radioiodide increased from 0.38 to 2.76 during a period of 34 days, while in Product A, batch No. 3 the percentage of free iodide increased from 1.49 to 4.62 during a period of 13 days. In Product D, batch No. 1, a small increase of free iodide from 0.07% to 0.64% could be observed during 14 days. A high specific activity (MBq/mg hippuran) accelerates the radiochemical decomposition (*16*). Since most manufacturers are stating only a range of specific activity of their products, the establishment of a relationship between specific activity and stability of the products is impossible. Product G contains AgCl-prepared ceramic particles to capture free radioiodide; a positive effect on the in vitro stability was not observed during our examinations.

Within an examining period of 1 mo, the two ^{125}I products showed only a slight increase in radiochemical impurity, while all three ^{123}I products had a significant increase in radiochemical impurity as early as 1 day after receipt. From viewing the test results in Table 3, it is apparent that the stability of $^{131}I/^{125}I/^{123}I$ hippurans essentially depends, as a function of storage time, on the physical half-life, type, and energy of radiation. Iodine-131 products (half-life 8.0 days, $E\gamma$ max = 364 keV) are subject to considerable decomposition whereas ^{125}I products (half-life 60.1 days, x-ray max = 27.5 keV) and ^{123}I products (half-life 13.2 hr, $E\gamma$ max = 159 keV) show only a negligible degree of decomposition. As shown in Table 3, all examined radiopharmaceuticals fulfill the requirements of the EP, regarding the content of *o*-iodobenzoic acid. With regard to the radiation dose, and especially because of the high thyroid exposure due to free iodide, the limit for this component has been set to 3%. This limit is exceeded by one manufacturer (Manufacturer A) out of those six that offer the ^{131}I products under examination. Products E, F, and G showed a percentage of iodide >2. The two ^{125}I products examined are below this value. None of the three examined ^{123}I products was found to exceed the value of 3% indicated for free iodide previous to the expiration date. The unknown radiochemical impurity that could be identified in all of the ^{131}I, ^{125}I, and ^{123}I hippurans examined, was found to have a higher percentage than *o*-iodobenzoic acid in several batches.

CONCLUSIONS

Radiochemical purity and in vitro stability are an indicator of the amount of the stated chemical form to the total radioactivity of the pharmaceutical, and also of the type and amount of radiochemical impurity. The causes for radiochemical impurities, among others, are the faulty chemical purity of the inactive initial product, the chemical instability of a compound and autoradiolysis. In cases where a radiopharmaceutical contains radiochemical impurities, the proper purpose of the product can be disturbed. Such impurities result in an undue radiation exposure to the patient and can affect the measurement of the organ under scrutiny by an increased background radiation from other regions of the body.

FOOTNOTES

* Schleicher & Schüll, Dassel, FRG.
† Berthold, Wildbad, FRG.

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