In Vivo Chemistry of Iofetamine HCl Iodine-123 (IMP)

Ronald M. Baldwin and Jiann-Long Wu

Medi-Physics, Inc., Emeryville, California

Application of chemical methods for characterizing the in vivo behavior of iofetamine HCl ^{123}I (IMP) has shed light on the metabolism of iofetamine in animals and humans. A successful technique consists of ethyl acetate extraction of the metabolites from tissue samples acidified with perchloric acid, separation of the mixture by high performance liquid chromatography, and quantitation of the radioactive components with a sensitive scintillation detector. Metabolism of iofetamine HCl ^{123}I proceeds sequentially from the N-isopropyl group on the amphetamine side chain. The first step, dealkylation to the primary amine p-iodoamphetamine (PIA), occurs readily in the brain, lungs, and liver; activity in the brain and lungs consists of only IMP and PIA even 24 hr after administration. The rate-limiting step appears to be deamination to give the transitory intermediate p-iodophenylacetone, which is rapidly degraded to p-iodobenzoic acid and conjugated with glycine in the liver to give the end product of metabolism, p-iodohippuric acid, which is excreted through the kidneys in the urine.

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With the increasing subtlety and sophistication of the techniques available in nuclear medicine today, it is becoming necessary not only to quantitate the amount of radioactivity in the different body compartments after administration of a radiopharmaceutical, but also to know the chemical form of the radioactivity. This is illustrated by iofetamine HCl iodine-123 (^{123}I N-isopropyl-p-iodoamphetamine hydrochloride, IMP), which has been amply demonstrated to distribute in brain tissue in a clinically useful fashion (*1,2*). However, the compound exhibits properties that suggest in vivo chemical transformations. The radioactivity level in the blood and plasma gradually increases over a period of 24–48 hr before clearing (*3–6*). Also, whereas initial distribution of ^{123}I in the brain corresponding to regional blood flow remains constant long enough for imaging, the relative distribution in the brain changes after several hours (*2,3*).

The objective of this paper is to review methods developed for characterizing the chemical behavior of iofetamine HCl ^{123}I in vivo as an example of measuring the pharmacokinetics of a radiopharmaceutical. The results of the measurement of IMP pharmacokinetics have been published (*6,7*). This review will focus on the special problems and advantages associated with pharmacokinetic analysis of radiopharmaceuticals.

Classically, a pharmacokinetic study concerns itself with five major processes (often termed the "LADME" system): liberation, absorption, distribution, metabolism, and elimination (*8*). For intravenously administered radiopharmaceuticals, liberation and absorption are essentially complete, and the gross distribution and elimination are readily determined by gamma camera imaging. The major goal of a pharmacokinetic investigation of a radiopharmaceutical is therefore to measure the metabolic products in vivo as a function of time. For IMP, the challenge lay in developing the methods for analyzing trace quantities of iofetamine HCl ^{123}I and its metabolites in body fluids.

Three metabolic reactions have been identified for IMP from animal experiments (*9–12*) and by analogy to metabolism of amphetamine (*13,14*): dealkylation, deamination, and oxidative degradation to iodobenzoic acid, followed by conjugation with glycine to form the major product of elimination, p-iodohippuric acid. Thus, the method had to detect IMP, p-iodoamphetamine, p-iodophenylacetone, p-iodobenzoic acid, and p-iodohippuric acid (Fig. 1).

The unique features of a radiopharmaceutical compared with ethical drugs result in both advantages and disadvantages. First, since the active ingredient is radioactive, the drug serves as its own tracer. On the other

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For reprints contact: Ronald M. Baldwin, Medi-Physics, Inc., 5855 Christie Ave., Emeryville CA 94608.

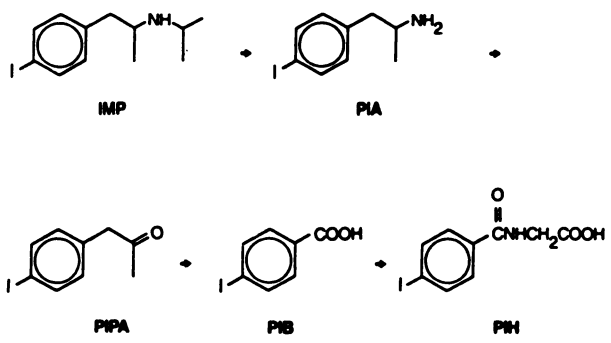

FIGURE 1
Proposed metabolic pathway for iofetamine.

hand, the effective sensitivity of detection decreases by a factor of two every half-life. Also, the very property that makes IMP so useful, the high tissue extraction, results in low plasma concentration. Human studies require exquisitely sensitive detection equipment because of the limitation in dose.

The task can be broken down into three problems: extraction of the metabolites from plasma and urine, HPLC separation, and detection of the components.

Metabolite Extraction

Extraction of the metabolites and treatment to make the samples amenable to the HPLC analysis requires special attention. Many materials are extensively conjugated in vivo (*15*) and exist in the circulation as esters with sulfate or cholesterol, or are extensively protein bound. Thus, after i.v. administration of iofetamine HCl ^{123}I to rats, simple extraction of the plasma with organic solvents did not remove all of the activity, and extra, polar, components were detected on HPLC of the extracts. A number of techniques have been proposed to circumvent the problem of conjugates in the plasma and urine. The simplest is to hydrolyze the esters by heating the solution in acid (*16*); such a vigorous treatment may also hydrolyze amide bonds, however. Enzymatic hydrolysis with a mixture of *beta*-glucuronidase and sulfatases may effect mild hydrolysis of conjugates without affecting other bonds (*17*); however, hydrolysis is often incomplete (*18*). The most effective technique for IMP was to treat the plasma with dilute perchloric acid and ethyl acetate. This brought about hydrolysis and also denatured the plasma proteins as a flocculent solid that was amenable to extraction with organic solvent. Ethyl acetate has a polarity high enough to extract even the relatively polar iodohippuric acid completely, and it seems to help in the gentle denaturation of serum proteins, so that after extraction, the solvent phase separates cleanly (*6*).

TABLE 1
Recovery of Metabolites from Body Fluids

	Extraction efficiency %	
Metabolite	Plasma	Urine
IMP	95.4	97.1
PIA	96.8	98.5
PIPA	90.6	97.1
PIB	96.1	98.4
PIH	97.5	98.6
I^-	39.6	0.1

All the components except iodide could be recovered in good yield from plasma and urine (Table 1). To ensure that individual metabolites were not selectively extracted from the biologic matrix, a known mixture of metabolites was added to the fluid and extracted. The ratio of individual metabolites (excluding iodide) was unchanged. Since iodide was incompletely extracted from plasma or urine under these conditions, iodide ion was determined by trapping it on a silver chloride filter bed, made by passing silver nitrate solution and normal saline through a paper filter (*19*). The trapping efficiency of ^{123}I iodide was 96.0%.

High Performance Liquid Chromatography Separation and Detection

The high performance liquid chromatography (HPLC) separation used paired ion chromatography (PIC) on a reversed phase column (*6*). Gradient elution with a gradient of the PIC reagent achieved baseline separation of the components in a reasonable elution time.

Individual metabolites were detected by their retention times on HPLC compared to known standards, and the concentration was quantitated by integrating the output of the radiochemical detector, which was calibrated against the concentration of ^{123}I in the fluid (plasma or urine), measured in a well counter. By comparing to an aliquot of the injected solution, the concentration could be calculated in terms of % injected dose.

The required sensitivity for detection on HPLC was achieved by passing the eluent through a coil contained in the cylindrical cavity of a scintillation crystal and analyzing the digital count data with a microcomputer (*6,20*). The detector response to ^{123}I was linear in the range of ~1 nCi to over 12 nCi, and amounts of ^{123}I as small as 0.3 nCi (12 Bq) could be detected, though not reliably quantified.

The stability of the metabolites was measured by HPLC after storage in blood, urine, and acetonitrile (the solvent for the samples awaiting HPLC). There was no indication of decomposition of the metabolites.

Metabolism of Iofetamine

The metabolic pathway of iofetamine HCl ^{123}I as determined in humans and animals is diagrammed in Figure 1. In the first step, iofetamine (IMP) is dealkylated to p-iodoamphetamine (PIA). This reaction occurs fairly rapidly, as seen by a peak of PIA concentration in human plasma ~8–12 hr after administration (*6*).

Animal experiments show that dealkylation occurs in brain, lungs, and liver (*9,10*). In rats, within 2 hr after administration, 20% of the IMP in the brain is converted to PIA, and after 24 hr, over 90% of the brain activity is present as the dealkylated primary amine PIA. The rest of the brain activity is unchanged IMP: no other metabolites have been found in this organ. A similar pattern was seen in the lungs.

The next step, which appears to be the rate-limiting step in metabolic degradation, is deamination, or scission of the C-N bond, leading to the ketone p-iodophenylacetone (PIPA). This intermediate does not accumulate, but is rapidly degraded further to p-iodobenzoic acid (PIB) by reactions that appear to occur mainly in the liver. The PIPA concentration in human plasma never exceeds 10% of the total plasma activity (*6*). PIB in turn is conjugated with glycine to p-iodohippuric acid (PIH), the end product of metabolism excreted in the urine. About 20% of the injected dose appears in the urine per day. There is no indication of in vivo deiodination (*6,9–10,21*), probably because of metabolism to the readily excreted PIB moiety before catabolism of the iodoaromatic ring can occur.

Applying the biochemical findings to the clinical picture, the increase in total plasma activity (*5,6*) can be explained by the release of metabolites into the plasma; after 6 hr, the major component of the plasma is p-iodobenzoate. "Redistribution" may be explained by the uptake of PIA, or perhaps IMP, released either from the brain or from other body pools. Animal experiments have shown that the brain uptake and retention of p-iodoamphetamine (PIA) is similar to that of iofetamine (*22,23*).

The case of iofetamine HCl ^{123}I illustrates how the application of chemical and pharmacokinetic techniques to radiopharmaceuticals completes the clinical picture, much as has been found for application of stable drugs.

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