Pharmacokinetic analysis of $^{123}$I-labeled medium chain fatty acid as a radiopharmaceutical for hepatic function based on beta-oxidation

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Beta-oxidation is the most important pathway to provide energy for the liver. Our recent findings indicated that radiolabeled medium chain fatty acid analogs could be used as radiopharmaceuticals in the liver, allowing us to monitor alterations in energy metabolism on the cellular level. In the present study, pharmacokinetic analysis of a radioiodinated medium chain fatty acid analog, $p$-$^{[123]}$Iiodophenylcyclohexanecarboxylic acid ($^{[123]}$IPEA), was carried out in normal and hepatitis model rats to investigate the index for the measurement of beta-oxidation activity in hepatocytes. The rate constant for metabolism of $^{[123]}$IPEA in the liver showed a strong correlation with the ATP level, which was determined as an indicator of beta-oxidation activity in hepatocytes. The radioactivity profile in the liver after $^{[123]}$IPEA administration provided important information regarding hepatic viability, and the metabolic rate constant of $^{[123]}$IPEA calculated by a pharmacokinetic method was a useful criterion for hepatic diagnosis based on hepatic cellular energy metabolism.

**Key words:** medium chain fatty acid, beta-oxidation, pharmacokinetic analysis, hepatic function, SPECT

**INTRODUCTION**

The development of an in vivo method for quantitative and regional assessment of hepatic viability is an important goal of clinical nuclear medicine. For this purpose, we recently developed $p$-$^{[123]}$Iiodophenylcyclohexanecarboxylic acid ($^{[123]}$IPEA) as a radiopharmaceutical for use in single photon emission computed tomography (SPECT) (Fig. 1).1 $^{[123]}$IPEA showed high initial uptake in the liver immediately after the injection. $^{[123]}$IPEA incorporated into the liver was metabolized via beta-oxidation, and its radiometabolites were rapidly eliminated from the liver into the urine. In addition, the rate of elimination of radioactivity from the liver was significantly delayed in hepatitis model rats compared with that in normal rats. Thus, in the present study, the hepatic time-activity curves obtained by dynamic scanning with a SPECT instrument were analyzed by a pharmacokinetic method in normal and hepatitis model rats, and some parameters were evaluated as the criteria for determining liver function.

**MATERIALS AND METHODS**

**Materials**

Sodium $^{[123]}$iodide was purchased from Daiichi Pure Chemical Co. Ltd., Chiba, Japan. Ammonium $^{[123]}$iodide was kindly provided by Nihon Medi-Physics Co., Hyogo, Japan. All chemicals were of reagent grade and were used as received.

**Synthesis of Radioiodinated IPEA**

IPEA was synthesized by iodiumination of 7-phenylheptanoic acid which was synthesized by Friedel-Crafts reaction from benzene and pimelic acid followed by reduction. Details of the synthetic procedure will be published.
elsewhere. [123I]IPEA and [125I]IPEA were prepared by the 127I123I or 127I125I exchange reaction with non-radioactive IPEA as a precursor. The radiochemical purity of each radiiodinated IPEA was more than 96% as determined by high performance liquid chromatography (HPLC) on a reverse-phase column (Cosmosil SC18-AR-300, 250 mm × 10 mm, Nacalai Tesque, Co., Ltd., Kyoto) with a mobile phase of acetonitrile/water (70/30, v/v) at 30°C (Rt. = 32 min).

**Animals**

Animal experiments were conducted in accordance with our institutional guidelines and were approved by the Kyoto University Animal Care Committee.

Male Wistar rats (200–250 g) were housed for 1 week under a 12-h light/12-h dark cycle and had free access to food and water. Hepatitis model rats were generated by administration of carbon tetrachloride (CCl4). Briefly, rats were orally administered 30% CCl4 (0.5–1.5 ml/kg CCl4/kg) in olive oil (v/v) after a 6-h fast. After fasting for a further 2 h, the animals were fed ad libitum.

**Metabolite Analysis**

Four normal rats anesthetized with pentobarbital were administered 74 kBq of [123I]IPEA via the tail vein. At 20 min post-injection, the rats were sacrificed by decapitation, and the liver was excised immediately. The total lipids in the liver samples were extracted with chloroform/methanol/water (4/2/2) as reported by Forch et al. The organic layer and water-soluble layer were separated, and radioactivity in each was quantified. Then the metabolites in the each layer were analyzed by TLC (reverse-phase TLC plate LKC14F, Whatman International Ltd., Kent, UK.) developed with acetonitrile/water/acetate (7/3/0.01).

**Dynamic Scanning of [123I]IPEA**

Imaging studies were performed in 12 rats with a SPECT instrument (SPECT2000H-40, Hitachi Medical Co., Japan). Normal rats and those treated with different doses of CCl4 were anesthetized with pentobarbital (50 mg/kg i.p.). Dynamic planar scanning (20 sec × 30 frames, 1 min × 10 frames, 2 min × 20 frames) was initiated at the time of [123I]IPEA (18 MBq) injection via the tail vein. Regions of interest (ROI) were chosen on the upper half of the liver images to eliminate the influence of radioactivity from the kidneys, and the time-activity curves were obtained after decay correction.

After scanning, liver samples of about 100 mg were excised from each rat, and the ATP concentration in the liver was measured according to the procedure reported by Sellevold et al. with minor modifications. Briefly, the liver sample was immediately frozen and powdered in liquid nitrogen. Then the ATP in the powdered liver was extracted with 3 ml of perchloric acid (0.42 M). After precipitation of proteins and neutralization with potassium hydroxide, ATP was quantified by HPLC on a reverse-phase column (Cosmosil SC18-AR-300, 150 mm × 4.6 mm, Nacalai Tesque, Co., Ltd., Kyoto) with a mobile phase containing potassium dihydrogen phosphate 215 mM, tetrabutylammonium hydrogen sulfate 2.3 mM, acetonitrile 10% and potassium hydroxide to adjust the pH to 6.0. The flow rate was maintained at 1.0 ml/min, and the spectrophotometer was set at 254 nm.

The 12 rats used in this study were divided into 3 groups according to the ATP concentration in the liver as follows: severe injury group, ATP levels of less than 80 μmol/100 g wet liver; moderate injury group, from 80 to 110 μmol/100 g wet liver; and normal group more than 110 μmol/100 g wet liver. Each of these 3 groups consisted of 4 rats.

**Data Analysis (Pharmacokinetic Model)**

Pharmacokinetic models were constructed based on the following assumptions: (i) [123I]IPEA in the blood was transported into hepatocytes monoexponentially; (ii) [123I]IPEA in the hepatocytes was metabolized by one-way metabolism; (iii) the radiometabolites in the hepatocytes were eliminated from the liver only into blood; and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Symbols used in the models</th>
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<tbody>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>X</td>
<td>[123I]IPEA in blood compartment</td>
</tr>
<tr>
<td>k4</td>
<td>Transport rate constant of [123I]IPEA from blood to liver</td>
</tr>
<tr>
<td>k5</td>
<td>Rate constant of elimination of [123I]IPEA by the other organs</td>
</tr>
<tr>
<td>X1</td>
<td>Unchanged [123I]IPEA in liver compartment</td>
</tr>
<tr>
<td>X2</td>
<td>Radiometabolites in liver compartment</td>
</tr>
<tr>
<td>A·e−k4t</td>
<td>Transport rate of [123I]IPEA from blood to liver</td>
</tr>
<tr>
<td>k2</td>
<td>Transport rate constant of unchanged [123I]IPEA from liver</td>
</tr>
<tr>
<td>k3</td>
<td>Metabolic rate constant in the liver</td>
</tr>
<tr>
<td>k4</td>
<td>Transport rate constant of radiometabolites from liver</td>
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<tr>
<th>Table 2</th>
<th>Mass balance for each compartment in the pharmacokinetic models shown in Figure 2</th>
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<tbody>
<tr>
<td>dX1/dt</td>
<td>X1 = − (k4 + k5) · X; X1(0) = X0</td>
</tr>
<tr>
<td>dX2/dt</td>
<td>A·e−k4t · X1 = − (k3 + k4) · X2; X2(0) = 0</td>
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Model 1

\[ \text{Blood} \xrightarrow{k_a} \text{Liver} \]

\[ \text{Blood} \xrightarrow{k_b} \text{Other Organs} \]

Model 2

\[ \text{Blood} \xrightarrow{A \cdot e^{-k_a \cdot t}} X_1 \xrightarrow{k_3} X_2 \xrightarrow{k_4} \text{Liver} \]

\[ k_2 \]

\[ k_1 \]

\[ k_0 \]

\[ k_3 \]

\[ k_4 \]

Fig. 2 Pharmacokinetic model used for the analysis of radioactivity profiles following intravenous injection of \( [^{123}]\text{IPEA} \) to rats.

Table 3 Pharmacokinetic parameters of \( [^{123}]\text{IPEA} \) in rats

<table>
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<tr>
<th></th>
<th>Normal</th>
<th>Moderate injury</th>
<th>Severe injury</th>
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<tbody>
<tr>
<td>( A )</td>
<td>416.0</td>
<td>395.1</td>
<td>310.9</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>3.931</td>
<td>3.811</td>
<td>2.984</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>0.0728</td>
<td>0.0461</td>
<td>0.0339</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>0.0754</td>
<td>0.0610</td>
<td>0.0502</td>
</tr>
<tr>
<td>( k_4 )</td>
<td>0.0042</td>
<td>0.0018</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

(iv) the radiometabolites in blood were not re-accumulated in hepatocytes.

Two pharmacokinetic models are depicted schematically in Figure 2. Symbols and the mass-balance equation are given in Tables 1 and 2, respectively. Model 1 represents clearance of \( [^{123}]\text{IPEA} \) from the blood of whole body. Model 2 represents hepatic uptake, metabolism and excretion. The constants \( k_a \) and \( k_b \) are the rate constants for liver uptake and elimination by the other organs, respectively. Eq. (1) and (2) were obtained from the mass-balance equation shown in Table 2, and Eq. (2) indicated that \( [^{123}]\text{IPEA} \) in the blood was transported into hepatocytes monoexponentially. Thus, when \( k_a \cdot X \) and \( k_0 + k_e \) were replaced by \( A \) and \( k_1 \), respectively, Eq (2) was simplified by \( A \cdot e^{-k_1 \cdot t} \) as the input function of \( [^{123}]\text{IPEA} \) to the liver.

\[ X = X_0 \cdot e^{-(k_0 + k_e) \cdot t} \]

\[ k_a X = k_0 X_0 \cdot e^{-(k_0 + k_e) \cdot t} = A \cdot e^{-k_1 \cdot t} \]

The following first-order constants were used in Model 2 (Fig. 2): \( k_2 \) for elimination of intact \( [^{123}]\text{IPEA} \) from hepatocytes; \( k_3 \) for metabolism of \( [^{123}]\text{IPEA} \) in hepatocytes; and \( k_4 \) for elimination of radiometabolites from hepatocytes. Solving the differential equations for Model 2 (Table 2) yields:

\[ X_1 = \frac{1}{k_1 - (k_2 + k_3)} \cdot \left(-A \cdot e^{-k_1 \cdot t} + A \cdot e^{-(k_2 + k_3) \cdot t}\right) \]

\[ X_2 = \frac{-k_3}{k_1 - (k_2 + k_3)} \cdot A \cdot e^{-k_1 \cdot t} + \frac{k_3}{k_1 - (k_2 + k_3)} \cdot \frac{k_3}{(k_4 - (k_2 + k_3))} \cdot A \cdot e^{-(k_2 + k_3) \cdot t} \]

\[ + \frac{k_3}{(k_4 - (k_2 + k_3))} \cdot A \cdot e^{-k_4 \cdot t} \]

\[ X_1 + X_2 = \frac{k_1 - (k_3 + k_4)}{k_1 - (k_2 + k_3)} \cdot A \cdot e^{-k_1 \cdot t} + \frac{k_4 - k_2}{k_1 - (k_2 + k_3)} \cdot \frac{k_3}{(k_4 - (k_2 + k_3))} \cdot A \cdot e^{-(k_2 + k_3) \cdot t} \]

\[ + \frac{k_3}{(k_4 - (k_2 + k_3))} \cdot A \cdot e^{-k_4 \cdot t} \]

Since it was impossible to distinguish radioactivity from intact \( [^{123}]\text{IPEA} \) and that from radiometabolites in the hepatocytes by external imaging, the information obtained from the hepatic images on radioactivity represents the sum of them \( (X_1 + X_2) \).

The equation for the amount of radioactivity in the liver \( (X_1 + X_2) \) (Eq. 5) was fitted to the radioactivity profiles obtained by dynamic scanning of \( [^{123}]\text{IPEA} \) by the nonlinear least-squares method (MULTI)\(^5\) and constants \( k_1 \), \( k_2 \), \( k_3 \), \( k_4 \) and \( A \) were determined.

RESULTS AND DISCUSSION

Figure 3 shows the mean time-activity profiles of the livers obtained by dynamic scanning of each group. These results are shown by the relative radioactivity in the liver in order to evaluate the clearance rate. The normal group showed a higher initial uptake and faster clearance in the liver than the CCl\(_4\)-treated group. Although about 44% of the maximum radioactivity uptake in the liver remained in the normal rats at 60 min after injection, this might be the result of the slower elimination from the liver of the radiometabolite rather than its production through beta-oxidation and/or of the relatively high background seen in the planar image. All of these time-activity profiles showed two phase clearance. Although the second phase clearance was not clearly different among these groups, the first phase clearance was delayed depending on the degree of injury due to CCl\(_4\). That is, at each clearance point in the severe injury group and after 5 min of moderate injury the portion of radioactivity remaining in the liver was significantly higher than that in the normal group.

Administration of CCl\(_4\) causes peroxidation of fat, which causes the collapse of systems enclosed by membranes including mitochondria.\(^6\)\(^7\) The enzymes involved in beta-oxidation are located in the mitochondrial matrix.\(^8\)
values for parameters in each group are shown in Table 3.

Metabolite analysis showed that 85.5% of radioactivity in the normal rat liver was derived from radiometabolites of $^{[125]I}$IPEA. This percentage matched the calculated $\frac{X_2}{X_1 + X_2} \times 100$ value for normal rats based on Eq. 5 (Table 4). Therefore, since the present pharmacokinetic model was considered appropriate, it was also applied to experimental data analyses for hepatitis model rats.

For further investigation on the index of beta-oxidation activity in hepatocytes, the time-radioactivity profile of each animal was fitted individually. The rate constant $k_3$ was considered to be related to beta-oxidation in hepatocytes based on Model 2. On the other hand, since beta-oxidation is the main pathway responsible for production of ATP in the hepatocytes, the level of ATP in the liver was regarded as an indicator of the beta-oxidation activity in hepatocytes. Therefore, the relationship between the first-order constant $k_3$ for the metabolic rate of $^{[125]I}$IPEA in hepatocytes and the ATP concentration in the liver was evaluated as shown in Figure 4. There was a good correlation between $k_3$ and the ATP level ($r = 0.769$). This indicated that $k_3$ is a potentially useful criterion which allows determination of the beta-oxidation activity in hepatocytes externally after $^{[125]I}$IPEA administration.

The metabolic rate via the beta-oxidation in the liver might be influenced by the anesthetic. But in order to eliminate the effect of the anesthetic, in all experiments in the present study the same anesthetic was used, and further study of the anesthetic may be required to elucidate its effect.

In conclusion, when $^{[125]I}$IPEA was administered to rats, the degree of injury in hepatocytes due to CCl$_4$ could be detected as the delay in radioactivity clearance from the liver. Furthermore, pharmacokinetic analysis indicated that the rate constant $k_3$ for metabolism of $^{[125]I}$IPEA could be used to quantify hepatocyte viability based on beta-oxidation activity.

REFERENCES


