Differential Pharmacokinetics of Acetohexamide in Male Wistar–Imamichi and Sprague–Dawley Rats: Role of Microsomal Carbonyl Reductase

Yorishige IMAMURA* and Hideaki SHIMADA

Abstract

Acetohexamide (AH) is a male-specific and androgen-dependent enzyme in rats. However, the male Wistar–Imamichi (WI) rat strain, unlike other strains such as male Wistar, Fischer 344 and Sprague–Dawley (SD) rats, lacks most of the microsomal carbonyl reductase, which is significantly smaller than that in male SD rats. Testectomy caused a marked decrease, from 105.5 ± 11.1 to 44.3 ± 11.8 ml/h/kg, in the Clp of AH in male SD rats. These results indicate that microsomal carbonyl reductase plays a critical role in the differential pharmacokinetics of AH in male WI and SD rats.

Key words acetohexamide; pharmacokinetics; microsomal carbonyl reductase; rat

Acetohexamide (AH) is an oral antidiabetic drug having a ketone group within its chemical structure. Ketone-containing drugs such as AH are reduced to their corresponding alcohol metabolites by an NADPH-dependent enzyme referred to as carbonyl reductase (EC 1.1.1.184). It has been reported that when AH is used as a model substrate, carbonyl reductase activity is detected both in subcellular fraction of liver microsomes and in the cytosol in rats. Interestingly, microsomal carbonyl reductase is a male-specific and androgen-dependent enzyme in rats. However, the male Wistar–Imamichi (WI) rat strain, unlike other strains such as male Wistar, Fischer 344 and Sprague–Dawley (SD) rats, lacks most of the microsomal carbonyl reductase in its liver microsomes. On the other hand, cytosolic carbonyl reductase activity is fully detectable in the liver of all tested rats, and cytosolic carbonyl reductase contributes to the pharmacokinetics of AH in all strains of male and female rats.

In addition to cytosolic carbonyl reductase, to elucidate whether microsomal carbonyl reductase can be involved in the pharmacokinetics of AH in male rats, it is useful to utilize male WI rats lacking most of microsomal carbonyl reductase. The purpose of the present study is to demonstrate the pharmacokinetic role of microsomal carbonyl reductase by comparing the plasma concentrations of AH after intravenous administration to male WI and SD rats.

MATERIALS AND METHODS

Materials

AH was supplied by Shionogi Co. (Osaka, Japan). Hydroxyhexamide was synthesized from acetohexamide according to the method of Girgis-Takla and Chrones. Other chemicals were of reagent grade.

Animals and Treatments

Male and female WI rats at 6 and 9 weeks of age were purchased from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan). Male and female SD rats at 6 and 9 weeks of age were obtained from Japan SLC (Shizuoka, Japan). All animal experiments were undertaken in compliance with the guidelines and procedures of Kumamoto University for the care and use of laboratory animals. Testectomy of male rats was performed at 6 weeks of age. The testectomized rats were raised under controlled lighting (12 h light/dark cycle), temperature (20 ± 2 °C) and humidity (50 ± 10%) up to 9 weeks of age. They were fed commercial rat chow and had free access to tap water.

Pharmacokinetic Experiments and Data Analysis

The animals at 9 weeks of age were lightly anesthetized with ether and cannulated in the jugular vein. AH dissolved in a solution of isotonic saline (0.15 % NaCl–0.1 NaOH–NaCl) was administered through the vein at a dose of 10 mg/kg body weight. Blood samples (0.25 ml each) were drawn at appropriate time intervals after AH administration, and centrifuged at 1500 g for 10 min to obtain plasma. The plasma concentration of AH was measured by the HPLC method. The elimination half-life of AH was determined by least-squares regression analysis of terminal log-linear portions of the plasma concentration–time profile. The plasma clearance (Clp) of AH was determined according to the following equation:

\[ Clp = \frac{D}{AUC} \]

where D is the dose and AUC is the area under the plasma concentration–time curve from zero to infinite time. The value of AUC was calculated using a microcomputer.

Statistical Analysis

Statistical analysis of the data was performed by Student’s t-test, and p < 0.05 was considered to be significant.

RESULTS

Strain Difference in Pharmacokinetics of AH
shows the plasma concentration–time profiles of AH after intravenous administration to male WI and SD rats. AH was eliminated more slowly from plasma in the WI than in the SD strain. The pharmacokinetic parameters ($t_{1/2}$, $AUC$ and $CLp$) of AH derived from the data are summarized in Table 1. The $CLp$ of AH (72.8±11.2 ml/h/kg) in male WI rats was significantly smaller than that (105.5±11.1 ml/h/kg) in male SD rats. In female WI and SD rats, the pharmacokinetic parameters of AH were also derived from the data for the plasma concentration–time profiles. However, no significant difference was observed between these AH parameters in female WI and SD rats (Table 1).

**Effect of Testectomy on Pharmacokinetics of AH** The effect of testectomy on plasma concentrations of AH after intravenous administration was examined in male WI and SD rats (Fig. 2). Table 2 summarizes the pharmacokinetic parameters ($t_{1/2}$, $AUC$ and $CLp$) derived from the data. Testectomy caused marked alterations in the pharmacokinetic parameters of AH in male SD rats. Interestingly, testectomy also had a significant effect on these parameters in male WI rats. Testectomized male WI and SD rats exhibited similar values in the pharmacokinetic parameters of AH.

**DISCUSSION**

Of the rat strains tested, the WI strain has several unique pharmacological and toxicological characteristics compared to other rat strains.11,12 One example is a strong resistance to the lethal toxicity of cadmium in male WI rats, suggesting a deficiency of transporters responsible for essential metals.11,13 In the present study, we confirmed that when AH was used as the substrate, microsomal carbonyl reductase activities in the liver and kidney of male WI rats were much lower than those in the liver and kidney of male SD rats (data not shown). On the other hand, cytosolic carbonyl reductase activities in the liver and kidney were not different between the WI and SD strains (data not shown). These results suggest that microsomal carbonyl reductase is involved in the pharmacokinetics of AH in male SD rats, but not in male WI rats. In fact, the $CLp$ of AH in male WI rats was significantly smaller than that in male SD rats. It should be noted that the role of microsomal carbonyl reductase in the pharmacokinetics of AH in male SD rats was demonstrated by using male WI rats, which lack most of the microsomal enzyme in the liver and kidney. In the present study, AH was given at a dose of 10 mg/kg body weight to male WI and SD rats. Additional studies may be necessary to confirm whether the role of microsomal carbonyl reductase in the pharmacokinetics of AH is affected by changing the dose.

Some products which are hydroxylated in the cyclohexyl ring of (−)-hydroxyhexamide, a major metabolite of AH, are known as urinary metabolites,1 although the detailed chemical structures of the hydroxylated metabolites remain to be identified. Thus, it is possible that a drug-metabolizing enzyme(s) other than microsomal carbonyl reductase is responsible for the hydroxylation of AH in male WI and SD rats. Cytochrome P450 (CYP) 2C11 exhibits broad versatility as an effective catalyst in the hydroxylation of a number of drugs and xenobiotics in male rats.14 For example, the hydroxylation of sulfamethazine and (+)-limonene is mainly catalyzed by CYP2C11.15,16 CYP2C11 is male-specific in

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**Table 1. Pharmacokinetic Parameters of AH in Male and Female WI and SD Rats**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Parameters</th>
<th>WI</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>$t_{1/2}$</td>
<td>1.98±0.32**</td>
<td>1.17±0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$AUC$ (μg·h/ml)</td>
<td>140±21**</td>
<td>96±10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$CLp$ (ml/h/kg)</td>
<td>72.8±11.2**</td>
<td>105.5±11.1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>$t_{1/2}$</td>
<td>4.15±0.71</td>
<td>3.73±0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$AUC$ (μg·h/ml)</td>
<td>323±27</td>
<td>354±27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$CLp$ (ml/h/kg)</td>
<td>31.1±2.7</td>
<td>28.4±2.4</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. of four to five rats. **p<0.01, significantly different from male SD.

**Table 2. Pharmacokinetic Parameters of AH in Testectomized Male WI and SD Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>WI</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>$t_{1/2}$</td>
<td>1.98±0.32</td>
<td>1.17±0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$AUC$ (μg·h/ml)</td>
<td>140±21</td>
<td>96±10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$CLp$ (ml/h/kg)</td>
<td>72.8±11.2</td>
<td>105.5±11.1</td>
<td></td>
</tr>
<tr>
<td>Tx</td>
<td>$t_{1/2}$</td>
<td>2.97±0.37**</td>
<td>2.98±0.92*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$AUC$ (μg·h/ml)</td>
<td>226±25***</td>
<td>238±64**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$CLp$ (ml/h/kg)</td>
<td>44±6.74**</td>
<td>44±3.1±11.8***</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. of three to five rats. U, untreated; Tx, testectomized. **p<0.05, **p<0.01, ***p<0.001, significantly different from untreated in the corresponding strain.
rats, and its expression is decreased by testectomy, indicating that the CYP isoform is an androgen-dependent enzyme.\textsuperscript{17–20} If the cyclohexyl ring of AH is hydroxylated by male-specific CYP2C11, testectomy will decrease the expression of CYP2C11 in addition to microsomal carbonyl reductase in male SD rats, whereas it will decrease the expression of only CYP2C11 in male WI rats. That is, not only in male SD rats, but also in male WI rats, the pharmacokinetics of AH will be affected by testectomy. As expected, testectomy caused different alterations between male WI and SD rats in the pharmacokinetic parameters of AH, and as a result, the testectomized male WI and SD rats exhibited similar values in these parameters.

The pharmacokinetic parameters of AH in testectomized male WI and SD rats approximated, but were not fully consistent with, those in female WI and SD rats. This may be because a male-specific but androgen-independent factor is also involved in the pharmacokinetics of AH in male rats. However, no information on such a factor is available so far. Further studies are in progress to elucidate the characteristics of male-specific drug-metabolizing enzymes and drug transporters responsible for the pharmacokinetics of AH in male rats.

In conclusion, the present study provides evidence that microsomal carbonyl reductase, which is male-specific and androgen-dependent, plays a critical role in the differential pharmacokinetics of AH in male WI and SD rats.

REFERENCES