EFFECTS OF DIETHYLIDITHIOCARBAMATE, A METABOLITE OF DISULFIRAM, ON THE PHARMACOKINETICS OF ALCOHOL AND ACETALDEHYDE IN THE RAT

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The effects of diethylidithiocarbamate (DDC), a metabolite of disulfiram which is known as Antabuse, on the blood concentrations of alcohol and acetaldehyde were determined simultaneously by head space gas chromatography in rats. After an intravenous injection of alcohol, the blood concentration of acetaldehyde was much lower than that of the alcohol. A pharmacokinetic model featuring the liver compartment for acetaldehyde was used to estimate pharmacokinetic parameters on the assumption that the distribution volumes of the central compartments were same for alcohol and acetaldehyde, and that the elimination rate of acetaldehyde from liver was large enough to isolate the liver compartment from the central compartment.

The results showed that the clearance of alcohol was 0.0226 l/min/kg and the elimination rate constant of acetaldehyde from the liver compartment was very large and 35 min⁻¹. The administration of DDC decreased the above significantly to 0.0132 l/min/kg and 20 min⁻¹, respectively.

After intravenous infusion of acetaldehyde, the time course of the blood concentration of acetaldehyde was analyzed by the one compartment model. The estimated elimination rate constants from blood and the distribution volume were in good agreement with those calculated from alcohol injection, indicating the appropriateness of the method used in this study. DDC had no effect on the elimination of infused acetaldehyde from blood indicating that the elimination may be due to the loss from lungs into breath, from skin surfaces and/or from the kidney but not by metabolism in the liver.

Keywords — diethylidithiocarbamate; disulfiram; Antabuse; alcohol; acetaldehyde; pharmacokinetic; head space gas chromatography

INTRODUCTION

After alcohol is consumed, the alcohol is oxidized to acetaldehyde in liver by an alcohol dehydrogenase. The acetaldehyde induces many unpleasant pharmacological effects such as flushing, headache, nausea, vomiting, vertigo and blurred vision. In clinical treatment of alcoholism, a disulfiram known as Antabuse is orally administered. It is immediately decomposed to diethylidithiocarbamate (DDC) in the body and inhibits the elimination of alcohol and acetaldehyde from blood. Patients who have been administered DDC suffer from unpleasant effects of acetaldehyde after drinking alcohol and come to dislike it. Since some cephalosporins which have a thiomethyltetrazole group in their structures show the Antabuse effect, special caution regarding the avoidance of alcohol should be given to patients.

Because the possible Antabuse effects of new cephalosporins exist, it is important to study the pharmacokinetic parameters of alcohol and acetaldehyde in experimental animals. In this report, these parameters and also the effect of DDC on these parameters were studied in rats.

MATERIALS AND METHODS

Materials — Sodium DDC (trihydrate) was
purchased from Wako Chemicals, Osaka, Japan. All other reagents were commercial products of the finest grade available.

Preparations for Gas Chromatography — Brown 3 ml vials with a rubber stopper and a plastic screw cap with a hole for needle insertion were used. The vials were prepared to contain 0.05 ml of internal standard solution (0.2% n-propanol) and 0.2 ml of 7% HClO₄ solution. Known amounts of alcohol and acetaldehyde were added to the vials to obtain calibration curves. To prepare acetaldehyde solutions, all fluids, apparatuses and syringes were refrigerated (4—6 °C) before use, and all dilutions were done in closed vials to prevent evaporation of acetaldehyde.

Animals — Male Sprague-Dawley rats weighing 250—300 g were used without fasting. The rats were anesthetized with sodium pentobarbital (5.5%, 0.8 ml/kg) and fixed on a board. Polyethylene tubings (PE 50, Clay Adams, U.S.A.) were used to cannulate the femoral arteries and the femoral veins. In DDC experiments, sodium DDC (0.0347 g/ml, 0.075 mmol/kg) was injected into the venous cannula 3 h before the alcohol injection or the acetaldehyde infusion.

Alcohol Injection — Isotonic alcohol solution (1.72 M, 5 ml/kg) was injected into the venous cannula. A blood sampling of 0.2 ml was done periodically from the arterial cannula using a heparinized syringe and the sample was injected immediately into a prepared vial.

Acetaldehyde Infusion — Isotonic acetaldehyde solution (0.03 μM) was infused into the venous cannula at a rate of 0.105 ml/min for 30 min by an infusion pump (JP-WG, Furue Sci., Tokyo). Blood samplings were taken periodically for 90 min during and after the infusion. In preliminary experiments, the infusion was done directly into a rubber stoppered vial to estimate the accuracy and constancy of the infusion rate and acetaldehyde concentration. From such vials, 99.5 ± 0.05% (mean ± S.D., n = 3) of added acetaldehyde was recovered.

Head Space Gas Chromatography — The determination of alcohol and acetaldehyde was done by a head space gas chromatography. The vial was immersed in a 60 °C water bath for 4 min, then 0.4 ml of the head space gas was drawn by a gas tight syringe that was kept at 60 °C by a water jacket. The condition of gas chromatography were as follows: Stainless steel column, 3 mm i.d. and 2 m long; column bed, Chromosolb 101; injection port temperature, 220 °C; column temperature, 130 °C; N₂ flow rate, 40 cm³/min; H₂ pressure, 0.6 kg/cm²; air pressure, 0.5 kg/cm²; instrument, Shimadzu GC-7APF with hydrogen flame ionization detector (FID). Under this condition, the retention times for acetaldehyde, alcohol and n-propanol were 2.0, 4.5 and 6.0 min, respectively.

Pharmacokinetic Parameter Estimation — In the case of alcohol injection, the fates of alcohol and acetaldehyde were determined by the pharmacokinetic model shown in Chart 1 a). The blood concentrations of alcohol (C₁) and acetaldehyde (C₄) are presented as followings:

![Chart 1](image)

**Chart 1. Pharmacokinetic Models for Alcohol and Acetaldehyde**

a) Alcohol injection and b) acetaldehyde infusion.

The subscripts i and j denote the compartment number, kᵢⱼ is the transport (from i to j) or elimination (i = j) rate constant, Vᵢ is the distribution volume and Cᵢ is alcohol or acetaldehyde concentration.
\[ C_1 = \frac{D}{V_1 (\lambda_1 - \lambda_2)} \left[ (\lambda_1 - k_{21}) e^{-\lambda_1 t} + (k_{21} - \lambda_2) e^{-\lambda_2 t} \right] \]

\[ = Ae^{-\lambda_1 t} + Be^{-\lambda_2 t} \]

\[ C_4 = \frac{Dk_{13}k_{34}}{V_4} \left[ \sum_{i=1}^{4} \frac{a_i}{\prod_{j \neq i}^{4} (\lambda_j - \lambda_i)} e^{-\lambda_i t} \right] \]

where \( D \) is the dose and \( \lambda_i \) is the rate constant. The areas under the blood concentration versus time curve (AUC) and the clearance of alcohol (Cl_{alc}) are given by

\[ AUC = \frac{A}{\lambda_1} + \frac{B}{\lambda_2} \]

\[ Cl_{alc} = \frac{D}{AUC} \]

When acetaldehyde was given by infusion over a period of \( T \) min, the one compartment model as shown in Chart 1 b) with only the 4th compartment was used. The blood concentration of acetaldehyde (\( C_4 \)) is expressed by Eqs. 6 and 7.

\[ 0 \leq t \leq T: \quad C_4 = \frac{k_0}{k_40 V_4} \left( 1 - e^{-k_{40} t} \right) \]

\[ T < t: \quad C_4 = \frac{k_0}{k_40 V_4} \left( 1 - e^{-k_{40} T} \right) e^{-k_{40} (t - T)} \]

where \( k_0 \) is the infusion rate, and the clearance of acetaldehyde (Cl_{alc}) is given by Eq. 8.

\[ Cl_{alc} = k_40 V_4 \]

The pharmacokinetic parameters were calculated by a nonlinear least-squares regression using the damping Gauss-Newton method. The weighting factors were the reciprocals of the data.

RESULTS

Alcohol Injection

Figure 1 shows the time courses of blood alcohol and acetaldehyde after intravenous injection of alcohol and the effect of DDC on them. It was noted that the blood concentrations of acetaldehyde were less than one percent of that of alcohol. DDC obviously inhibited the elimination of alcohol and acetaldehyde from blood. The slope of the terminal phase of the time course of blood acetaldehyde appears to be similar to that of alcohol.

FIG. 1. Effect of DDC on Blood Concentrations of Alcohol and Acetaldehyde after Alcohol Injection

a) Control and b) DDC treated. Key: ——○——, ——△—— and ———□—— represent the individual rat. Lines are simulated using the parameters obtained from curve fitting.
TABLE I.  Pharmacokinetic Parameters of Alcohol$^{a)}$

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>$\lambda_1$</th>
<th>$\lambda_2$</th>
<th>$\lambda_{31c}$</th>
<th>$AUC$</th>
<th>$k_{12}$</th>
<th>$k_{21}$</th>
<th>$k_{13}$</th>
<th>$V_1$</th>
<th>$V_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.0</td>
<td>13.3</td>
<td>0.555</td>
<td>0.0388</td>
<td>0.0226</td>
<td>386</td>
<td>0.270</td>
<td>0.229</td>
<td>0.0943</td>
<td>0.243</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td>±4.5</td>
<td>±2.0</td>
<td>±0.144</td>
<td>±0.0019</td>
<td>±0.0043</td>
<td></td>
<td>±68</td>
<td>±0.110</td>
<td>±0.025</td>
<td>±0.0239</td>
<td>±0.019</td>
</tr>
<tr>
<td>DDC</td>
<td>23.8</td>
<td>14.7</td>
<td>0.576</td>
<td>0.0248</td>
<td>0.0132</td>
<td>679</td>
<td>0.303</td>
<td>0.239</td>
<td>0.0590</td>
<td>0.222</td>
<td>0.273</td>
</tr>
<tr>
<td></td>
<td>±2.1</td>
<td>±1.1</td>
<td>±0.402</td>
<td>±0.0072</td>
<td>±0.0032</td>
<td></td>
<td>±185</td>
<td>±0.239</td>
<td>±0.161</td>
<td>±0.0129</td>
<td>±0.006</td>
</tr>
<tr>
<td>p$^{b)}$</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td></td>
<td>p&lt;0.1</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p&lt;0.1</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

$^{a)}$ Values are means ± S.D. of three experiments.  $^{b)}$ Level of significance of difference.  N.S. = not significant.

TABLE II.  Pharmacokinetic Parameters of Acetaldehyde after Alcohol Injection$^{a)}$

<table>
<thead>
<tr>
<th></th>
<th>$k_{34}$</th>
<th>$\lambda_1$</th>
<th>$\lambda_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.436±0.115</td>
<td>35.0±0</td>
<td>0.954±0.732</td>
</tr>
<tr>
<td>DDC</td>
<td>0.508±0.151</td>
<td>20.0±1.0</td>
<td>0.678±0.198</td>
</tr>
<tr>
<td>p$^{b)}$</td>
<td>N.S.</td>
<td>p&lt;0.01</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

$^{a)}$ Values are means ± S.D. of three experiments.  $^{b)}$ Level of significance of difference.  N.S. = not significant.

of alcohol in each rat.

Compartment Analysis

The blood concentrations of alcohol were analyzed by the two compartment open model using Eq. 2 to obtain the parameters shown in Table I.

The blood concentrations of acetaldehyde after alcohol injection were analyzed by Eq. 3 with four exponential terms on the assumption that the distribution volume of acetaldehyde ($V_0$) is identical to that of alcohol ($V_1$). The alcohol parameters ($\lambda_1$, $\lambda_2$ and $k_{31c}$) of each rat were used to calculate acetaldehyde parameters ($\lambda_3$, $\lambda_4$ and $k_{34}$) for the same rat. The calculated parameters were averaged and are shown in Table II. The parameters in Tables I and II were used in the calculations to obtain the simulated lines shown in Fig. 1.

Acetaldehyde Infusion

The time course of the blood concentration of acetaldehyde during the infusion with acetaldehyde for 30 min and post-infusion are shown in Fig. 2. The data were analyzed by the one compartment model using Eqs. 6 and 7, and the cal-

![FIG. 2. Time Courses of Blood Concentration of Acetaldehyde after Acetaldehyde Infusion](image)

Key: ——— O———, control; ——— ○———, DDC treated.

Results of three rats for control and DDC treated, respectively, are plotted.

Lines are simulated using averaged parameters obtained from curve fitting.
**TABLE III.** Pharmacokinetic Parameters of Acetaldehyde after Acetaldehyde Infusion$^a$)

<table>
<thead>
<tr>
<th></th>
<th>$k_{40}$ (min$^{-1}$)</th>
<th>$V_4$ (l/kg)</th>
<th>$Cl_{ald}$ (l/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.956 ± 0.317</td>
<td>0.165 ± 0.068</td>
<td>0.143 ± 0.001</td>
</tr>
<tr>
<td>DDC</td>
<td>0.649 ± 0.102</td>
<td>0.229 ± 0.047</td>
<td>0.146 ± 0.012</td>
</tr>
<tr>
<td>$p$)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

$^a$) Values are means ± S.D. of three experiments. $^b$) Level of significance of difference. N.S. = not significant.

culated parameters are shown in Table III. DDC appeared to have no effect.

**DISCUSSION**

In the consumption of alcohol, many unpleasant effects are attributable to acetaldehyde, but little is known about the pharmacokinetics of acetaldehyde.$^4$) The precise determination of acetaldehyde concentration in blood is difficult due to the rapid elimination of acetaldehyde from blood, the low blood concentration of acetaldehyde and its low vaporizing point of 21 °C at 1 atm. In this study, evaporation of acetaldehyde was minimized by using refrigeration and closed systems. The time course of blood alcohol, after alcohol injection, was simulated by the proposed two compartment open model. DDC administration decreased the clearance of alcohol by nearly one half of the control but did not affect $k_{12}$, $V_1$ and $V_2$. The blood concentrations of acetaldehyde were much lower than those of alcohol. The reasons were thought to be as follows; hypothesis (a) the distribution volume of acetaldehyde is larger than that of alcohol, hypothesis (b) the fraction of alcohol which is oxidized to acetaldehyde is very small, hypothesis (c) the elimination of acetaldehyde from the body is more rapid than that of alcohol.

The acetaldehyde data after alcohol injection were analyzed by the two compartment model on the assumption that the elimination of acetaldehyde from liver was rapid that the liver was isolated as a compartment and the distribution volume of the blood compartment of acetaldehyde was the same as that of alcohol. Because the time courses of blood acetaldehyde were governed by those of alcohol, the alcohol parameters for each rat were used to calculate the acetaldehyde parameters for the same rat, resulting in the decrease of inter-individual deviations. The simulated lines with calculated parameters agreed with the observed acetaldehyde data after alcohol injection. The apparent large variance of the data with the simulated line in the low acetaldehyde concentrations was due to the semilogarithmic plot. The result shows that the rate constant ($\lambda_3$) regarding the compartment 3 was very large (35 min$^{-1}$) and DDC decreased it significantly to 20 min$^{-1}$. The $\lambda_3$ and $\lambda_4$ are expressed in terms of the individual rate constants as

$$\lambda_3 + \lambda_4 = k_{34} + k_{43} + k_{30} + k_{40}$$

The results show that $\lambda_4$, $k_{34}$ and $k_{40}$ were less than 1 min$^{-1}$, and $k_{43}$ may be negligible compared to $k_{40}$ as will be discussed later, and $\lambda_3$ approximates $k_{30}$ which is the elimination rate constant from the liver compartment. The large rate constants may reflect the proximity of the acetaldehyde production from alcohol to the enzymes or to the microsomes that rapidly metabolize acetaldehyde. This proximity will minimize the rate limiting steps such as the blood flow or the transport of the acetaldehyde molecules to the enzymes. These rate limiting steps are inevitable in a systemic acetaldehyde administration.

The results of acetaldehyde infusion show that the clearance of acetaldehyde was large and was not affected by DDC. This fact suggests that the transport of acetaldehyde from blood to
liver may be negligible compared to the elimination from blood. This rapid elimination of acetaldehyde may be due to expiration from the lung, evaporation from skin surfaces and loss by the renal excretion. The elimination rate constants from blood \((k_{40})\) were in a good agreement with the rate constant \((\lambda_4)\) obtained after alcohol injection. The distribution volumes \((V_i)\) were in a reasonable agreement with those of alcohol \((V_j)\). Thus the hypothesis (a) becomes unlikely. Since it is known that 90—98% of alcohol was oxidized to aldehyde,\(^1\) then hypothesis (b) is unlikely and hypothesis (c) may be correct.

The oxidative metabolism of alcohol and acetaldehyde in the liver compartment was simulated by a linear kinetic even though the metabolism is essentially a nonlinear process especially the inhibition by DDC. The use of the linear kinetic was reasonable since the results of all the data agreed with the simulated lines obtained with the parameters calculated by the linear compartment model, even in case of DDC experiments. The dehydrogenases for alcohol and acetaldehyde exist in many organs such as plasma and liver, and in many subcellular structures, such as cytosol, mitochondria and microsome.\(^5\) DDC inhibits only a portion of these enzymes leaving considerable amounts to metabolize alcohol and acetaldehyde.

In conclusion, the pharmacokinetical analysis of the blood concentrations of alcohol and acetaldehyde in the rat using the compartment model was successfully developed. The compartment model for acetaldehyde was characterized by rapid elimination from blood and rapid metabolism in liver. Using this method, a quantitative and qualitative determination for the Antabuse effects of any compound on alcohol and acetaldehyde will be possible in the rat.

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**REFERENCES**