EFFECT OF FUROSEMIDE AND TRIMETHAZIDINE ON KINETIC BEHAVIOR AND HYPOTENSIVE EFFECT OF HYDRAZALINE IN RATS

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The pharmacokinetic and pharmacodynamic interactions between hydralazine (HP) and furosemide or trimethazidine were determined following single i.v. and repeated (7 d) oral administrations in rats. Both furosemide and trimethazidine caused a significant decrease in the level of plasma protein binding of HP in vivo after i.v. administration. However, in vitro there was no effect on binding. Coadministration of HP and furosemide at lower doses (1.67 mg/kg, i.v. and 5 mg/kg, oral) reduced the hypotensive effect, accompanying the enhanced elimination of HP from plasma after a single i.v. and repeated oral treatment when compared to the result obtained with HP alone. The elimination of HP accelerated by the lower doses of furosemide may be probably due to the increase in renal clearance by the diuretic effect of furosemide. On the contrary, a high dose (10 mg/kg, i.v.) of furosemide temporarily strengthened the hypotensive effect, probably due to the additional hypotensive action of furosemide itself. Trimethazidine (0.75 and 3.75 mg/kg) gave a small increase in the hypotensive effect of HP in spite of the partially enhanced clearance of HP. The pharmacodynamic analysis showed that the hypotensive effect of HP depended upon the plasma HP concentration in each treatment, although the response curves of HP were partly altered by the combined drugs. The present study also suggests a validity of co-administration of HP and diuretics for the therapy of hypertension and heart failure.

Keywords — hydralazine; hydralazine-furosemide interaction; hydralazine-trimethazidine interaction; hydralazine pharmacodynamics; plasma level-hypotensive effect relationship

INTRODUCTION

Hydralazine (1-hydrazinophthalazine, HP), an arterial vasodilator, has been used extensively in the treatment of hypertension. The combination therapy with HP and furosemide or trimethazidine is common in the treatment of hypertension and heart failure. Current clinical experience indicates that when HP is used in combination with a diuretic to decrease fluid retention, satisfactory control of blood pressure is achieved for many patients.

Many reports on alterations of a drug action by other drugs have appeared in the literature. McLean et al. have reported that oral HP results in a peak propranolol concentration 2 times higher than that without HP in 7 healthy volunteers and Nomura et al. have shown that HP affects furosemide kinetics in patients with congestive heart failure. Therefore, a possibility of interaction between HP and diuretics should be considered in the therapy of hypertension. However, there is little information concerning the disposition and the pharmacological effect of HP when used in concurrent therapy. Additionally, the relationship between antihypertensive response and plasma HP concentration is ambiguous, although we found a correlation between the plasma HP concentration and the magnitude of the hypotensive effect after concurrent intraperitoneal injection of HP and phenobarbital in normotensive and spontaneously hypertensive rats. Thus, it is of great interest to clarify the pharmacokinetic and pharmacodynamic interaction of HP and diuretics when they are coadmni-
nistered.

The purpose of this study, using normotensive rats, was to examine the influence of furosemide and trimethazidine on the pharmacokinetics and pharmacodynamics of HP after a single intravenous (i.v.) injection and repeated oral treatment and to estimate, preliminarily, the validity of coadministration of HP and these diuretics.

MATERIALS AND METHODS

Materials — Hydralazine hydrochloride (HP) and 1-hydrazino-4-methylphthalazine, an internal standard for gas-liquid chromatography (GLC), were kindly supplied by Japan Ciba-Geigy Co., Ltd. Furosemide and trimethazidine dihydrochloride were gifts from Yodogawa Pharm. Co., Ltd. and Toa-Eiyo Co., Ltd., respectively. Other chemicals were of the highest grade commercially available.

Treatment of Animals — Male Wistar rats (150—200 g) were used throughout and allowed free access to food (MF diet, Oriental Yeast Co., Ltd.) and water prior to the experiments.

a) Single Intravenous Administration: HP (5 mg/kg) alone or in combination with furosemide (1.67 and 10 mg/kg) or trimethazidine (0.75 and 3.75 mg/kg) was prepared in saline and administered intravenously (i.v.) into a tail vein (0.1 ml/100 g body weight), but furosemide was dissolved in a small amount of dimethylformamide and diluted with saline.

b) Repeated Oral Administration: HP (7.5 mg/kg) alone or in combination with furosemide (5 and 10 mg/kg) or trimethazidine (0.75 and 3.75 mg/kg) was prepared as aqueous suspensions in 2% acacia and administered in a volume of 0.5 ml/100 g body weight for 7 d.

Determination of HP in Plasma — Blood samples (about 0.15 ml) following final treatment with drug(s) were collected periodically in heparinized syringes from a jugular vein of unanesthetized rats. The blood was immediately centrifuged at 1 500 × g at 4 °C and plasma was separated. The plasma concentrations of HP were determined according to the GLC method of Zak et al., a specific analytical method for HP.

Measurement of Blood Pressure — Blood pressure was measured according to the tail pulse pick-up method previously described.4)

Determination of Free HP Concentration in Plasma — Animals were treated with a single i.v. injection of HP (7.5 mg/kg) alone or in combination with furosemide (1.67 and 10 mg/kg) or trimethazidine (3.75 mg/kg) into a jugular vein. Blood specimens (3 ml) were collected from the opposite jugular vein 0.75 and 3 h after dosing, respectively. The in vivo protein binding of HP was estimated by means of ultrafiltration based on centrifugation for 30 min at 4 °C in a dialysis bag (8/32 Visking tubing) containing one ml of plasma.6)

In Vitro Protein Binding of HP to HSA — Equilibrium dialysis technique was used to determine the binding of HP (1 × 10⁻⁴ M) in the presence and absence of furosemide (5 × 10⁻⁶ — 2 × 10⁻³ M) or trimethazidine (5 × 10⁻⁷ — 2 × 10⁻⁴ M) to human serum albumin (HSA, 5.2 × 10⁻⁵ M) by using the apparatus described by Goto et al.7) The dialysis apparatus was shaken (100 oscillation/min) on a Toyo incubator (TC-1) for 12 h at 20 °C under shaded light. The detailed experimental procedure is described in a previous paper.8)

Pharmacokinetic Analysis — In a single i.v. dosing, the plasma HP concentration data for individual animals were fitted to the equation:

\[ C_t = Ae^{-\alpha t} + Be^{-\beta t} \]

where \( C_t \) is the drug concentration at time \( t \). Pharmacokinetic constants (Table I) were determined from the biexponential equation constants, i.e., \( A, \alpha, \), \( B \) and \( \beta \), using conventional equations.9) The elimination of HP in rats were analyzed by a two-compartment model accompanied with the elimination from central compartment.

Since the half-life of HP is extremely short, the accumulation of HP in plasma during multiple dosing was negligible. Thus, in repeated oral administrations, a one-compartment model
including a first-order absorption process according to the Eq. 2 was used to describe the data:

\[ C_t = \frac{F \cdot \text{dose}}{V_d} \frac{k_a}{k_a - k_{el}} (e^{-k_{el}t} - e^{-k_at}) \]  

(2)

where \( k_a \) is the absorption rate constant and \( F \) is the fraction of drug absorbed.

All plasma level data, in the form of the total concentration of HP, were analyzed by the non-linear iterative least-squares regression analysis, MULTI.\(^{10}\) The parameters determined by the stripping method\(^{11}\) by graphical presentation were used as starting values, and the data obtained were weighted numerically equal.

The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal method up to the last sampling point \( (C_n) \) and the area to infinite time, which was less than 15% of the total area except for a single i.v. dose of HP alone (about 40%), was added by integrating \( (C_n/\beta \) or \( C_n/k_{el} \)). Total body clearance \( (C_{tot}) \) was estimated by the Eq. 3.

\[ C_{tot} = \frac{\text{dose}_{i.v.}}{AUC_{i.v.}} \]  

(3)

**Pharmacodynamic Analysis** — The concentration–response curves, relating total plasma concentration of HP to hypotensive effect, were expressed as the difference in blood pressure before and after drug administration and analyzed by computer fitting (MULTI)\(^{10}\) to the following equation:\(^{12}\)

\[ E = m \log C + e \]  

(4)

where \( E \) is the response, \( C \) is the plasma concentration of HP and \( m \) and \( e \) are the constants.

**Statistical Analysis** — Results are expressed as the mean ± S.D. Statistical analysis was performed using the non-paired Student's \( t \)-test and a \( p \)-value less than 0.05 was considered to be significant.

**RESULTS**

**Effect of Furosemide or Trimethazidine on Plasma Concentrations of HP after Single i.v. Administration**

The plasma concentration–time curves for HP in all groups declined biexponentially after dosing (Fig. 1), although two rats coadministered furosemide (1.67 mg/kg) were subjected to a

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**FIG. 1. Plasma HP Concentrations Following Single i.v. Administration of HP Alone or in Combination with Furosemide or Trimethazidine**

Lines are the calculated plasma HP concentrations using the linear two-compartment model and plots are the experimental data expressed as the mean ± S.D. of 5–10 rats.  
(a) ●, HP alone (5 mg/kg); ○, HP plus furosemide 1.67 mg/kg; ▲, HP plus furosemide 10 mg/kg.  
(b) ●, HP alone; ○, HP plus trimethazidine 0.75 mg/kg; ▲, HP plus trimethazidine 3.75 mg/kg.
TABLE I. Pharmacokinetic Parameters for HP Following Single i.v. Administration of HP Alone or in Combination with Furosemide (F) or Trimethazidine (TMZ)

<table>
<thead>
<tr>
<th></th>
<th>HP alone</th>
<th>+ F 1.67&lt;sup&gt;a,e&lt;/sup&gt;</th>
<th>+ F 10&lt;sup&gt;b&lt;/sup&gt;</th>
<th>+ TMZ 0.75&lt;sup&gt;c&lt;/sup&gt;</th>
<th>+ TMZ 3.75&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (µg/ml)</td>
<td>1.79 ± 0.43</td>
<td>1.10 ± 0.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.18 ± 0.43&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.84 ± 1.52</td>
<td>2.54 ± 0.94</td>
</tr>
<tr>
<td>α (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.08 ± 0.87</td>
<td>2.94 ± 0.85&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.20 ± 0.45</td>
<td>4.40 ± 1.08</td>
<td>4.30 ± 1.30</td>
</tr>
<tr>
<td>B (µg/ml)</td>
<td>2.13 ± 0.45</td>
<td>2.51 ± 0.35&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.00 ± 0.16</td>
<td>2.83 ± 0.63</td>
<td>2.66 ± 0.51</td>
</tr>
<tr>
<td>β (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.162 ± 0.035</td>
<td>0.398 ± 0.181&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.198 ± 0.043&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.426 ± 0.127&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.368 ± 0.102&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.19 ± 0.37</td>
<td>0.77 ± 0.41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.01 ± 0.34</td>
<td>1.69 ± 0.89</td>
<td>1.74 ± 1.05</td>
</tr>
<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.77 ± 0.61</td>
<td>2.13 ± 0.40&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.08 ± 0.20</td>
<td>2.33 ± 0.51</td>
<td>2.27 ± 0.45</td>
</tr>
<tr>
<td>k&lt;sub&gt;10&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.284 ± 0.044</td>
<td>0.464 ± 0.133&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.306 ± 0.093&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.816 ± 0.108&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.678 ± 0.101&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;1&lt;/sub&gt; (l/kg)</td>
<td>1.42 ± 0.24</td>
<td>1.59 ± 0.15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.60 ± 0.20&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.07 ± 0.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.11 ± 0.27&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;2&lt;/sub&gt; (l/kg)</td>
<td>0.96 ± 0.27</td>
<td>0.53 ± 0.13&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.76 ± 0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.66 ± 0.27&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.73 ± 0.33&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;0&lt;/sub&gt; (l/kg)</td>
<td>2.23 ± 0.41</td>
<td>2.03 ± 0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.35 ± 0.21&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.64 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.74 ± 0.24&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>AUC (µg/ml·h)</td>
<td>13.81 ± 1.41</td>
<td>7.53 ± 2.80&lt;sup&gt;h&lt;/sup&gt;</td>
<td>10.77 ± 1.48&lt;sup&gt;h&lt;/sup&gt;</td>
<td>7.71 ± 2.47&lt;sup&gt;h&lt;/sup&gt;</td>
<td>7.78 ± 1.63&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;tot&lt;/sub&gt; (l/h·kg)</td>
<td>0.365 ± 0.046</td>
<td>0.751 ± 0.273&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.475 ± 0.073&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.702 ± 0.230</td>
<td>0.680 ± 0.156&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5—10 rats. a) HP plus furosemide 1.67 mg/kg. b) HP plus furosemide 10 mg/kg. c) HP plus trimethazidine 0.75 mg/kg. d) HP plus trimethazidine 3.75 mg/kg. e) Two rats which were subjected to a monoexponential term were calculated by the equation: C<sub>t</sub> = B e<sup>−β·t</sup>. f) p < 0.05, g) p < 0.01 and h) p < 0.001, respectively, compared to HP alone.

monoexponential term. The pharmacokinetic parameters of HP obtained are shown in Table I.

Coadministration of HP and furosemide (1.67 mg/kg) or trimethazidine (0.75 and 3.75 mg/kg) enhanced the disappearance of HP from plasma. However, at a higher dose of furosemide (10 mg/kg) its effect on the elimination of HP decreased or became almost negligible. After coadministration with a 1.67 mg/kg dose of furosemide, the terminal elimination rate constant (β) was significantly increased compared to that of HP alone. Coadministration of trimethazidine at the dose of 0.75 and 3.75 mg/kg also resulted in a marked increase in the β value.

The AUC was significantly decreased after a concurrent administration of furosemide or trimethazidine compared to that of HP alone, resulting in a significant enhancement of C<sub>tot</sub>, with the exception of the C<sub>tot</sub> after coadministration at a 0.75 mg/kg dose of trimethazidine.

Effect of Furosemide or Trimethazidine on Plasma Concentrations of HP after Repeated Oral Administrations

Animals were subjected to daily (7 d) treatment with HP alone or in combination with furosemide or trimethazidine and plasma concentrations of HP were measured following the final administration. The α phase could not be detected after oral administration, as shown in Fig. 2. The pharmacokinetic parameters of HP after repeated treatment with the drug alone or in combination with furosemide or trimethazidine are listed in Table II. When HP was coadministered with 5 mg/kg of furosemide, the k<sub>el</sub> was significantly higher than that for HP alone, but a higher dose (10 mg/kg) of furosemide apparently did not alter the disposition of HP. Parameters other than the k<sub>el</sub> however were not changed by furosemide at both doses. On the other hand, the AUC was significantly reduced by trimethazidine (3.75 mg/kg) coadministered with HP, without a significant effect on the k<sub>el</sub>.

Effect of Furosemide or Trimethazidine on Blood Pressure

The effect of the combined drugs on rat blood pressure up to 4.5 h after a single i.v. administration is depicted in Fig. 3. After a single i.v. dose, the hypotensive effect of HP clearly decreased following coadministration with 1.67 mg/kg of furosemide. On the other hand, when a
Interaction of Hydralazine and Diuretics

FIG. 2. Plasma HP Concentrations Following Repeated Oral Administrations of HP Alone or in Combination with Furosemide or Trimethazidine

Lines and plots are the calculated and experimentally observed plasma HP concentrations, respectively. Each point represents the mean ± S.D. of 4—5 rats. (a) ●, HP alone (7.5 mg/kg); ○, HP plus furosemide 5 mg/kg; ▲, HP plus furosemide 10 mg/kg. (b) ●, HP alone; ○, HP plus trimethazidine 3.75 mg/kg.

TABLE II. Pharmacokinetic Parameters for HP Following Final Administration in Repeated Oral Treatment of HP Alone or in Combination with Furosemide or Trimethazidine

<table>
<thead>
<tr>
<th></th>
<th>HP alone</th>
<th>+ F 5a)</th>
<th>+ F 10b)</th>
<th>+ TMZ 3.75c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{el}$ (h$^{-1}$)</td>
<td>0.636 ± 0.212</td>
<td>1.137 ± 0.101 d)</td>
<td>0.757 ± 0.125</td>
<td>0.630 ± 0.157</td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>3.15 ± 1.76</td>
<td>1.38 ± 0.27</td>
<td>2.85 ± 1.52</td>
<td>4.14 ± 1.60</td>
</tr>
<tr>
<td>$t_{max}$ (h)</td>
<td>0.70 ± 0.18</td>
<td>0.80 ± 0.06</td>
<td>0.71 ± 0.23</td>
<td>0.55 ± 0.11</td>
</tr>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>0.213 ± 0.057</td>
<td>0.204 ± 0.074</td>
<td>0.282 ± 0.053</td>
<td>0.206 ± 0.039</td>
</tr>
<tr>
<td>$AUC$ (µg/ml·h)</td>
<td>0.553 ± 0.073</td>
<td>0.508 ± 0.092</td>
<td>0.657 ± 0.125</td>
<td>0.456 ± 0.056c)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4—5 rats. a) HP plus furosemide 5 mg/kg, b) HP plus furosemide 10 mg/kg, c) HP plus trimethazidine 3.75 mg/kg, d) $p < 0.01$ and e) $p < 0.05$, respectively, compared to HP alone.

10 mg/kg of furosemide was coadministered with HP, the hypotensive effect from 1.5 to 2.25 h after dose was significantly stronger when compared to that after HP administration alone (Fig. 3a). There was no significant difference in the hypotensive effect between HP alone and the HP plus trimethazidine groups.

In repeated oral administrations, the concurrent dose of HP and 5 mg/kg of furosemide tended to slightly reduce the hypotensive effect compared to that of HP alone, while a higher dose of furosemide (10 mg/kg) scarcely altered the effect. There was a general trend for the effect to be slightly enhanced in the trimethazidine coadministered group (Fig. 4).

It appears that the effect of combined drugs on the hypotensive effect is not very pronounced except for furosemide at a dose of 1.67 mg/kg. Effect of Furosemide and Trimethazidine on Protein Binding of HP

The effect of coadministered drugs on protein binding of HP was estimated. Table III shows the in vivo plasma protein binding of HP at 0.75 and 3 h after i.v. injection of HP alone (7.5
FIG. 3. Blood Pressure—Time Course Following Single i.v. Administration of HP Alone or in Combination with Furosemide or Trimethazidine

Each point represents the mean ± S.D. of 4—5 rats. (a) ●, HP alone (5 mg/kg); ○, HP plus furosemide 1.67 mg/kg; ▲, HP plus furosemide 10 mg/kg. (b) ●, HP alone; ○, HP plus trimethazidine 0.75 mg/kg; ▲, HP plus trimethazidine 3.75 mg/kg. a) $p < 0.05$ in HP alone vs. HP plus furosemide 10 mg/kg, b) $p < 0.025$, c) $p < 0.01$ and d) $p < 0.005$ in HP plus furosemide 1.67 mg/kg vs. HP plus furosemide 10 mg/kg. e) $p < 0.005$ in HP alone vs. HP plus furosemide 1.67 mg/kg.

FIG. 4. Blood Pressure—Time Course Following Repeated Oral Administrations of HP Alone or in Combination with Furosemide or Trimethazidine

Each point represents the mean ± S.D. of 4—5 rats. (a) ●, HP alone (7.5 mg/kg); ○, HP plus furosemide 5 mg/kg; ▲, HP plus furosemide 10 mg/kg. (b) ●, HP alone; ○, HP plus trimethazidine 3.75 mg/kg. a) $p < 0.025$ and b) $p < 0.01$ respectively compared to HP alone.

mg/kg) or in combination with furosemide (1.67 or 10 mg/kg) or with trimethazidine (3.75 mg/kg). The dose of HP was increased from 5 to 7.5 mg/kg since the concentration of unbound HP in plasma was too low to be detected at the 5 mg/kg dose.
### TABLE III. Effect of Furosemide and Trimethazidine on the in Vivo HP Binding

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.75 h</th>
<th>3.0 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Free&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP alone</td>
<td>2.640 ± 0.260</td>
<td>0.109 ± 0.017</td>
</tr>
<tr>
<td>HP + F 1.67</td>
<td>3.064 ± 0.539</td>
<td>0.238 ± 0.082&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP + F 10</td>
<td>2.632 ± 0.323</td>
<td>0.267 ± 0.098&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP + TMZ 3.75</td>
<td>2.923 ± 0.433</td>
<td>0.255 ± 0.062&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are expressed as μg/ml plasma (n = 3—6).<br>
<sup>b</sup> p < 0.05 and<sup>c</sup> p < 0.01, respectively, compared to HP alone.<br>
<sup>d</sup> Not detectable.

HP (7.5 mg/kg) was administered intravenously either alone or in combination with furosemide (F, 1.67 and 10 mg/kg) or trimethazidine (TMZ, 3.75 mg/kg). Blood was collected 0.75 and 3 h after administration.

Both drugs when coadministered caused a significant increase in the plasma concentration of free (unbound) HP at 0.75 h after dose, although there was no significant difference at 3 h after dose, the plasma concentration of free HP for the groups receiving two drugs tended to be slightly higher than that of the group receiving HP alone. The free fraction of HP was considered to be concentration-independent in the concentration range (0.221—2.640 μg/ml) observed in this study.

The effect of furosemide and trimethazidine on the binding of HP to HSA was also estimated in vitro. Furosemide (5 × 10<sup>-6</sup> — 2 × 10<sup>-3</sup> M) and trimethazidine (5 × 10<sup>-7</sup> — 2 × 10<sup>-4</sup> M), concentrations which are partly equivalent to the levels

![Graph](image)

**FIG. 5. Relationship between Plasma HP Concentration and Response Following Single i.v. Administration**

Response was expressed as the difference of blood pressure before and after drug administration. Each point represents the mean value of each group and lines are the calculated values using the linear least squares regression. (a) ●, HP alone (5 mg/kg); ○, HP plus furosemide 1.67 mg/kg; ▲, HP plus furosemide 10 mg/kg. (b) ●, HP alone; ○, HP plus trimethazidine 0.75 mg/kg; ▲, HP plus trimethazidine 3.75 mg/kg.
TABLE IV. Pharmacodynamic Parameters for HP Following Single i.v. Administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>m</th>
<th>e</th>
</tr>
</thead>
</table>
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 84.5 ± 15.8 | 26.8 ± 2.8 |
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 43.1 ± 6.7  | 33.3 ± 1.3 |
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 89.3 ± 9.8  | 37.9 ± 1.4  |
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 94.5 ± 30.4 | 21.4 ± 6.5 |
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 46.7 ± 10.7 | 38.3 ± 2.5 |
| HP alone  
| HP + F 1.67  
| HP + F 10  
| HP alone  
| HP + TMZ 0.75  
| HP + TMZ 3.75 | 50.1 ± 10.0 | 37.7 ± 2.4 |

The values were obtained from the least linear squares regression analysis.  
a) The data were obtained from the HP–furosemide interaction study.  
b) HP plus furosemide 1.67 mg/kg.  
c) HP plus furosemide 10 mg/kg.  
d) The data were obtained from the HP–trimethazidine interaction study.  
e) HP plus trimethazidine 0.75 mg/kg.  
f) HP plus trimethazidine 3.75 mg/kg.  
g) p < 0.05 and h) p < 0.001, respectively, compared to HP alone.

in human plasma, had no apparent effect on the in vitro protein binding of HP (1 × 10^{-4} M). The bound fraction of HP was 62.3 ± 0.4% in the absence of the diuretics, and 59.4 ± 2.2 or 58.4 ± 2.1% in the presence of furosemide or trimethazidine, respectively.

Relationship between Total Plasma HP Concentration and Hypotensive Effect after Coadministration with Furosemide or Trimethazidine

The response (the difference of blood pressure before and after dosing) curves of HP after a single i.v. administration are shown in Fig. 5 as a function of logarithmic total plasma concentration. The parameters calculated by Eq. 4 are presented in Table IV.

The hypotensive effect was dependent upon the logarithmic total plasma HP concentration in each group, and there was a correlation between the response and concentration (r = 0.874–0.977). However, the response curves were altered by an i.v. dose of both furosemide and trimethazidine. The larger dose (10 mg/kg, i.v.) of furosemide caused the parallel shift of the response curve to the left. With a lower dose (1.67 mg/kg, i.v.) of furosemide the curve was clearly altered at the lower concentrations of

![Figure 6](image)

**FIG. 6. Relationship between Plasma HP Concentration and Response Following Repeated Oral Administrations**

Each point represents the mean value of each group and lines are the calculated values using the linear least squares regression.  
(a) ●, HP alone (7.5 mg/kg); ○, HP plus furosemide 5 mg/kg; ▲, HP plus furosemide 10 mg/kg.  
(b) ●, HP alone; ○, HP plus trimethazidine 3.75 mg/kg.
TABLE V. Pharmacodynamic Parameters for HP Following Repeated Oral Administrations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>m</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP alone</td>
<td>26.3±5.2</td>
<td>46.0±5.1</td>
</tr>
<tr>
<td>HP + F 5 a)</td>
<td>19.8±1.7</td>
<td>38.3±1.8</td>
</tr>
<tr>
<td>HP + F 10 b)</td>
<td>17.5±1.7</td>
<td>40.5±1.5</td>
</tr>
<tr>
<td>HP + TMZ 3.75 c)</td>
<td>33.6±5.0</td>
<td>63.3±5.6 d)</td>
</tr>
</tbody>
</table>

The values were obtained from the least linear squares regression analysis.  

a) HP plus furosemide 5 mg/kg.  
b) HP plus furosemide 10 mg/kg.  
c) HP plus trimethazidine 3.75 mg/kg.  
d) p < 0.001 compared to HP alone.

HP: the slope of the curve was significantly decreased, 84.5±15.8 to 43.1±6.7 mmHg·ml·μg⁻¹. However, the pharmacodynamic behavior in the higher HP concentration ranges, in the case of coadministration with the low dose of furosemide, was no longer distinguishable from that of HP alone.

On the other hand, the concentration-response curve of HP shifted to the left with both doses of trimethazidine in the lower HP concentration range, but was not significant (0.05 < p < 0.1). The magnitude of curve shift by trimethazidine was independent of the dose of trimethazidine.

The concentration-response curves following repeated oral dosing are shown in Fig. 6. Furosemide, at both doses, did not apparently affect the total plasma concentration-response relation of HP; the regression equation for the combined data in HP-furosemide experiments was E = 15.7 (±4.5) log C + 37.1 (±4.7). On the contrary, the curve was significantly shifted to the left after coadministration with trimethazidine.

DISCUSSION

The disappearance of HP from plasma was enhanced by both a lower dose of furosemide (1.67 mg/kg, i.v. or 5 mg/kg, oral) and i.v. trimethazidine (Figs. 1 and 2), while an increased dose (10 mg/kg) of furosemide relatively reduced the elimination of HP. The increased Cltot of HP by furosemide or trimethazidine can probably be ascribed to the potent diuretic effect and increased blood flow, and the increase in the free fraction of HP by these drugs (Table III) may partly contribute to the enhanced elimination of HP. However, the slightly slower elimination of HP after a high dose of furosemide in both single i.v. and repeated oral treatments, compared to that in a lower dose of furosemide, cannot be explained rationally in view of the present results obtained. Perhaps, the pharmacokinetic interaction may be counterbalanced by the dramatically opposite action of furosemide at high doses, such as the contraction of extracellular fluid volume and change in electrolyte balance, the increased blood flow, sodium retention and decreased urine volume induced by HP. Iwaki et al. have reported that when the hemoconcentration was induced by a high dose of furosemide (50 mg/kg, i.p.) in rats, the inulin clearance and the fractional excretion of uric acid significantly decreased.

A low dose (1.67 mg/kg, i.v.) of furosemide decreased the hypotensive effect (Fig. 3) compared to that of HP alone. This can be explained by the pharmacokinetic interaction containing the increased clearance of HP. The slightly enhanced antihypertensive effect of HP when coadministered with trimethazidine (Fig. 3) may be in part related to the increased free fraction of HP in plasma which temporally induces an increment of hypotensive action. Additionally, the strengthened hypotensive activity at 1.5–2 h after a high dose of furosemide is due to the hypotensive effect of furosemide itself and partly to the increased free fraction of HP. Consequently, these effects affect the concentration–response
relation, apparently resulting in a shift to the left (Fig. 5). The reason why an additive effect did not occur, at 0.75 h after i.v. dose of 10 mg/kg furosemide, may be due to the fact that HP at 5 mg/kg i.v. dose exerted the maximum hypotensive effect immediately after dosing under the conditions tested and an additive decrease in blood pressure by furosemide was not possible. Judging from the results obtained, the increased free fraction of HP may contribute to the partial increment of hypertensive effect in the coadministration with trimethazidine, while in the combination of furosemide the increased free level appears to be related to the increased elimination of HP. With the exception of few data, furosemide appears to exert a potent diuretic effect\textsuperscript{18} whereas trimethazidine does not.\textsuperscript{18}

The present study shows that furosemide and trimethazidine caused a reduction of the in vivo protein binding of HP during a short time after administration (Table III), but did not in vitro. The differences in the effect of combined drugs on protein binding of HP and in the percentage protein binding of HP in the in vivo and in vitro system were perhaps due to the difference in protein concentrations (the concentration in vitro was $5.2 \times 10^{-5}$ M, 0.343%). When the in vitro protein concentrations were diluted, the bound fraction of drugs would be significantly altered.

There was a significant difference in the pharmacodynamics of HP between single i.v. and repeated oral administrations (Figs. 5 and 6). This difference may be due to the drastic differences of plasma concentrations between the two administration routes (Figs. 1 and 2). The plasma levels after oral administration were much lower than those after an i.v. dose, probably due to the poor bioavailability,\textsuperscript{19} resulting in a smaller response and thus giving a gently slope of the response-concentration curve (Fig. 6).

Ludden et al.\textsuperscript{20} reported that the time course of hypotension produced by HP is not directly related to the plasma HP concentration at a given time. However, Zacest et al.\textsuperscript{21} shows a good correlation between plasma HP level and hypotensive action. The lack of a temporal relationship between response and plasma concentration is due to at least partly to the fact that many of the methods described above were non-selective for HP and metabolites and that the circulating and inactive metabolites are thus partly estimated as HP. The analytical method used for this study was more selective than many of the methods described above. The dynamic relationship between the plasma HP level and hypotensive effect obtained in our study (Figs. 5 and 6) clearly demonstrated that the hypotensive effect depended primarily on the plasma concentration, although the free levels of HP in plasma and the pharmacological effect of combined drug itself should be considered. The shift of the total plasma concentration-response curves after coadministration might be at least partially due to the increased free HP concentration in plasma. Additionally, the shift of curves after a higher dose of furosemide appears to be ascribed to the antihypertensive effect of furosemide itself. In fact, a 10 mg/kg dose (i.v.) of furosemide alone induced some hypotensive effect from 0.75 to 2.25 h after administration (data were not shown), but trimethazidine did not.

In conclusion, a lower dose of furosemide reduced the hypotensive effect of HP accompanying with the enhanced disappearance of HP from plasma. However, the combination of HP and an appropriate dose of diuretics is thought to be valid therapeutically based on the result of the present experiments using rats. Furthermore, the estimation of plasma HP levels, which correlated roughly with hypotensive effect, makes it possible to predict the pharmacodynamic analysis, although the concentration-response curves were slightly influenced by these combined drugs.

REFERENCES
2) A. J. McLean, H. Skews, A. Bobik and F. J. Dudley: Interaction between oral propranolol and hydralazine,
Interaction of Hydralazine and Diuretics


