Kinetic Studies of the Pharmacologic Response to Captopril in Rats. II. 1) Hypotensive Effect and Plasma Angiotensin Converting Enzyme Activity

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(Received June 6, 1988)

The effect of captopril on the mean arterial blood pressure was studied in rats. Two different disease-state rats, namely the sodium-deficient rat (SDR) and the two-kidney-one-clip Goldblatt hypertensive rat (GHR), as well as normotensive rats (NR), were used. After i.v. bolus administration of captopril to each rat, the time course of plasma angiotensin converting enzyme (ACE) activity and mean arterial blood pressure were determined. In a different experiment, the effect of captopril on the plasma ACE activity in vitro was determined. Captopril inhibited the plasma ACE activity in a concentration-dependent manner and the relationship between concentration of captopril and inhibition of plasma ACE activity in vitro was reasonably described by a Langmuir-type equation. Then, plasma concentrations of captopril after i.v. administration were estimated by means of this equation. The estimated plasma concentration of captopril followed a double exponential equation. From the data obtained, a kinetic model including the renin-angiotensin system and pharmacokinetics of captopril was constructed under the following assumptions: (1) the hypotensive effect of captopril is solely attributable to the reduction of angiotensin II level in the body, (2) the production rate of angiotensin II is proportional to the total ACE activity and (3) plasma ACE activity reflects the total ACE activity in the body. Then, the effect of captopril on the mean arterial blood pressure in each type of rat was calculated. The results indicated that the hypotensive effect of captopril in NR was reasonably described by the model. However, the hypotensive effect of captopril in both GHR and SDR could not be well described by the model. These facts indicate that the decrease in mean arterial pressure in response to captopril may be due to a combination of decreased circulating levels of angiotensin II and an angiotensin-independent mechanism such as the kallikrein-kinin system, at least in the high-renin condition.

Keywords — captopril; angiotensin converting enzyme; mean arterial pressure; pharmacokinetics; pharmacodynamics; renal hypertensive rat; sodium deficient rat

Introduction

Captopril is an inhibitor of angiotensin converting enzyme (ACE) and lowers arterial blood pressure in various kinds of hypertensive patients. 2) Inhibition of ACE decreases circulating levels of angiotensin II (Ang II) and aldosterone, and increases the level of bradykinin in the body. 3) All of these factors are considered to be effective to decrease the mean arterial blood pressure (MAP). Numerous reports on the hypotensive effect of captopril have been published; however, the precise mechanism is still unclear. On the other hand, the pharmacokinetics of captopril have been investigated extensively in several animals including humans. 4) As far as we are aware, few reports have appeared on the quantitative relationship between the pharmacokinetics and pharmacodynamics of captopril.

In the previous study, 1) we elucidated the renin-angiotensin system in different disease-state rats, using a kinetic model. The purposes of this investigation were to clarify the pharmacokinetic-pharmacodynamic relationship of captopril after i.v. administration, and to verify the applicability of the previous model to the pharmacologic effect of captopril in rats.

Materials and Methods

Chemicals — Captopril was kindly supplied
by Sankyo Co. (Tokyo, Japan). Benzoyl-
glycyl-L-hystidyl-L-leucine (HHL) was obtained
commercially from the Peptide Institute, Inc.
(Minoh, Japan). All other chemicals were of
analytical grade and were used without further
purification.

**Animals** — Male Wistar rats were supplied
by Shizuoka Laboratory Animal Center (Hamamatsu,
Japan). Two kinds of disease-state rats,
namely two-kidney-one-clip Goldblatt renal hy-
pertensive rats (GHR) and sodium-deficient
rats (SDR) were employed as described previ-
ously. GHR and SDR as well as normotensive
rats (NR) were used throughout this study.
These rats were implanted with cannulas into
the left jugular vein and the left temoral artery,as
reported previously.

**Determination of the Relationship between
Plasma Captopril Concentration and ACE Ac-
tivity** — Pooled plasma from 3 to 4 rats in each
case was used to determine the inhibitory effect
of captopril on ACE in *vitro*. Aliquots of capto-
pril were added to the blank plasma to make
final concentrations of 0.001 to 10 μg/ml. Then,
plasma ACE activity was determined by using
HHL as the substrate.

**Determination of the Pharmacologic Effect
of Captopril** — Captopril was dissolved in 5%
dextrose solution and administered intravenously
at dose levels of 1 to 10 mg/kg. The time
courses of mean arterial blood pressure (MAP)
and plasma ACE activity were determined.
Details of the experimental procedures and con-
ditions, including the method of data analysis,
were described in the previous paper.

**Results**

(1) **In Vitro** Plasma ACE Activity

The relationship between captopril concen-
tration and plasma ACE activity in the three
type of rats is shown in Fig. 1, where plasma
ACE activity is expressed as the relative value,
obtained by dividing the ACE activity with capto-
pril by the respective control (without capto-
pril) ACE activity. The greater the concentration
of captopril, the less ACE activity was observed.

If captopril inhibits the plasma ACE activity
in a non-competitive manner, the following
equation can be derived:

\[
\frac{dy}{dt} = \frac{V(S_o - y)}{K_S + (S_o - y)} \cdot \frac{1}{(1 + \frac{I}{K_1})}
\]  

(1)

where \( y \) represents the amount of hippuric acid
produced by the reaction and \( S_o \) represents the
initial amount of substrate (HHL). \( V \) and \( K_S \)
are the maximum rate and the Michaelis con-
stant, respectively. \( K_1 \) and \( I \) represent the disso-
ciation constant and the concentration of capto-
pril as a non-competitive inhibitor, respectively.
Under the present experimental conditions, \( S_o \)
was sufficiently large compared to \( K_S \) and \( y \), so
Eq. 1 reduces to Eq. 2.

\[
\frac{dy}{dt} = \frac{VK_1}{K_1 + I}
\]  

(2)

By integrating Eq. 2, the amount of hippuric
acid produced by the reaction at time \( t \) is given
by Eq. 3.

\[
y = \frac{VK_1}{K_1 + I} t
\]  

(3)

Under the control condition (without addition
of captopril), Eq. 3 reduces to Eq. 4.

\[ y_0 = Vt \]  

(4)

Since relative plasma ACE activity \( f_A \) is defined as \( y/y_0 \), \( f_A \) is simply given by Eq. 5.

\[ f_A = \frac{K_1}{K_1 + I} \]  

(5)

The lines shown in Fig. 1 represent the calculated values according to Eq. 5, and the estimated parameter \( K_1 \) is listed in Table I. The \( K_1 \) value of GHR was much smaller than that of NR or SDR. This fact indicates that the sensitivity to captopril of plasma ACE activity was increased by the disease state.

(2) Effect of Captopril on the Plasma ACE Activity

Time courses of the plasma relative ACE activity after captopril administration in the three different types of rats are shown in Fig. 2 (symbols). Some of the data for NR and SDR were taken from the previous report. The relative

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**Table I. Pharmacologic Parameter of ACE Activity in Vitro**

<table>
<thead>
<tr>
<th></th>
<th>NR Estimate ± S.D.</th>
<th>SDR Estimate ± S.D.</th>
<th>GHR Estimate ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_1 ) ( \mu g/ml )</td>
<td>0.219 ± 0.043</td>
<td>0.299 ± 0.050</td>
<td>0.053 ± 0.017</td>
</tr>
</tbody>
</table>

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Fig. 2. Time Course of Relative ACE Activity after i.v. Administration of Captopril in Rats

Each value is the mean ± S.E. Most of the S.E. values were smaller than the size of the symbols. The lines represent theoretical values according to Eq. 11 in the text. Upper, middle and lower plots are the 1, 5 and 10 mg/kg studies, respectively.

(A) NR 1 mg/kg (n = 2), 5 mg/kg (n = 3), 10 mg/kg (n = 2). (B) SDR 1 mg/kg (n = 7), 5 mg/kg (n = 5), 10 mg/kg (n = 3). (C) GHR 1 mg/kg (n = 5), 5 mg/kg (n = 4), 10 mg/kg (n = 4).
plasma ACE activity showed a prominent decrease just after the administration and returned gradually to the respective premedication levels thereafter, regardless of the disease state. If Eq. 5 is also applicable to the relationship between the plasma relative ACE activity and plasma captopril concentration \textit{in vivo}, theoretical values for the plasma captopril concentration after i.v. administration could be obtained by applying the following equation.

\[ C_p = \frac{f_A}{f_A} \frac{1}{K_1} \]  \hspace{1cm} (6)

where \( C_p \) is the plasma concentration of captopril after i.v. administration.

The points shown in Fig. 3 are the estimated values for the plasma captopril concentrations, given as the dose-normalized data. The time course of the estimated plasma concentrations showed a double exponential decline, so these data were fitted to the following equation.

\[ C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \]  \hspace{1cm} (7)

Where \( C_1 \) and \( C_2 \) are constants and \( \lambda_1 \) and \( \lambda_2 \) are rate constants of the double exponential equation. The results are shown in Fig. 3 as the lines, and the mean values of the estimated parameters are listed in Table II. The time courses of the estimated plasma concentrations of captopril could be described reasonably well by a double exponential equation.

\textbf{(3) Effect of Captopril on the Blood Pressure}

The effect of captopril on the mean arterial blood pressure (MAP) in NR, SDR and GHR is

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
 & NR & SDR & GHR \\
\hline
\( C_1 \), \( \mu g/ml/mg/kg \) & 0.884 \( \pm \) 0.279 & 0.838 \( \pm \) 1.044 & 0.334 \( \pm \) 0.094 \\
\hline
\( C_2 \), \( \mu g/ml/mg/kg \) & 0.0716 \( \pm \) 0.0179 & 0.420 \( \pm \) 0.231 & 0.114 \( \pm \) 0.060 \\
\hline
\( \lambda_1 \), \( h^{-1} \) & 5.33 \( \pm \) 2.04 & 5.89 \( \pm \) 6.48 & 3.80 \( \pm \) 2.16 \\
\hline
\( \lambda_2 \), \( h^{-1} \) & 0.494 \( \pm \) 0.147 & 0.203 \( \pm \) 0.155 & 0.362 \( \pm \) 0.148 \\
\hline
\end{tabular}
\caption{Pharmacokinetic Parameters of Captopril Estimated from the Plasma ACE Activity\textsuperscript{a)}
}
\end{table}

\textsuperscript{a)} Each value (\( \pm \) S.E.) was obtained by the standard two stage (STS) method of Sheiner and Beal.\textsuperscript{90}
Fig. 4. Effect of Captopril on the Mean Arterial Blood Pressure in Rats

Each value is the mean ± S.E. Most of the S.E. values were smaller than the size of the symbols. The lines represent theoretical values according to Eq. 10 in the text. Upper, middle and lower plots are the 1, 5 and 10 mg/kg studies, respectively.

(A) NR 1 mg/kg (n = 4), 5 mg/kg (n = 4), 10 mg/kg (n = 3). (B) SDR 1 mg/kg (n = 6), 5 mg/kg (n = 6), 10 mg/kg (n = 3). (C) GHR 1 mg/kg (n = 4), 5 mg/kg (n = 4), 10 mg/kg (n = 5).

shown in Fig. 4 (symbols). Some of the data for NR and SDR were taken from the previous report.5) In NR, MAP was decreased just after the administration and returned to the premedication value in a dose-dependent manner. In SDR, MAP also decreased just after the administration, but did not return to the premedication value within the experimental period, especially in the high dosage studies (5, 10 mg/kg). In GHR, the pattern of the effect was similar to that in SDR, but the hypotensive effect was much greater.

In the previous paper,1) we elucidated the renin-angiotensin system in rats. The amount of Ang II in the body at the steady-state was described by Eq. 8.

\[
A_2 = \frac{R \cdot k_{12}}{(k_{12} + k_{a1}) k_{a2}}
\]  

where \( A_2 \) is the concentration of Ang II and \( R \) is the zero-order intrinsic production rate of angiotensin I (Ang I). \( k_{a1} \) and \( k_{a2} \) are the first-order rate constants of Ang I and II, respectively. \( k_{12} \) is the first-order rate constant for the conversion of Ang I to Ang II at the control (premedication) state. After i.v. administration of captopril, plasma ACE activity was reduced according to Eq. 5, in this study. It is reasonable to consider that the total ACE activity in the body was inhibited by captopril in the same ratio as plasma ACE activity. Then, substituting \( k_{12} f_A \) for \( k_{12} \) in Eq. 8 and rearranging yields Eq. 9.

\[
A_2 = \frac{R \cdot f_A}{k_{a1}/k_{12} + f_A} = \frac{k_{a2}}{k_{a2}}
\]
Consequently, MAP during captoril administration can be written as Eq. 10.\(^1\)

\[
\text{MAP} = \text{MAP}_0 + \frac{V_{\text{max}} \left( \frac{R \cdot f_A}{k_{a1}/k_{12} + f_A} \right)^r}{(K_m \cdot k_{a2})^r + \left( \frac{R \cdot f_A}{k_{a1}/k_{a2} + f_A} \right)^r}
\]

where MAP\(_0\) is the mean arterial pressure controlled by the homeostatic system other than the renin-angiotensin system. \(V_{\text{max}}\) is the maximum pressor response to Ang II. \(K_m\) and \(r\) are the constants of the Hill’s equation. Details of these parameters were given in the previous report.\(^1\)

By substituting captoril concentration, according to Eq. 7, into Eq. 5, the following equation is obtained.

\[
f_A = \frac{K_1}{K_1 + C_1 e^{-\lambda_i t} + C_2 e^{-\lambda_j t}}
\]

(4) Calculation of the Pharmacological Response to Captoril

The effect of captoril on the plasma ACE activity at each dose and in each type of rat was calculated by using Eq. 11, and the results are shown in Fig. 2, as the lines. The parameter values used in the calculation were taken from Tables I and II. Each calculated value described the experimental data fairly well.

MAP after captoril administration was also calculated by using Eq. 10 and the results are shown in Fig. 4, as the lines. The values for the parameters MAP\(_0\), \(R\), \(k_{a1}/k_{12}\), \(K_m\), \(k_{a2}\), \(r\) and \(V_{\text{max}}\) were taken from the previous report. The calculated values for MAP after captoril administration to NR at each dose described the experimental data reasonably well. In both SDR and GHR, the duration and the pattern of the hypotensive effect were well explained by the model; however, there were some discrepancies between calculated values and observed data in the recovery phase of MAP. In the 1 mg/kg dose study, an overshooting phenomenon was observed at the recovery phase and it could not be explained by the model. At higher dosages (5, 10 mg/kg), each observed value for MAP was always smaller than the theoretical prediction.

Discussion

In the present study, the time course of plasma concentration of captoril after i.v. administration to each type of rat was not determined directly. The hypotensive effect of captoril is attributable, at least in part, to the inhibitory effect on ACE activity. Therefore, the data for the time course of the ACE activity after captoril administration may provide better information concerning the hypotensive effect than the plasma or urine concentration of the intact drug. Since the pharmacologic effect of captoril on the plasma ACE activity was considered to be a direct effect, as suggested by Figs. 1 and 2, the pharmacokinetics of captoril in plasma could be estimated by the method of Smolen and Weigand.\(^7\) Thus, the “estimated” plasma concentrations of captoril, shown in Fig. 3, were used in a manner identical to data derived from the direct assay for the drug in plasma, in this study.

Due to its free sulphydryl group, captoril in plasma readily binds to albumin and other plasma proteins and forms mixed disulfides with endogenous thiol-containing compounds.\(^{4a}\) Since these disulfide metabolites can be reduced back to intact captoril by a number of enzymatic and chemical reactions,\(^{4a}\) the metabolism and disposition of captoril have been difficult to elucidate. As pointed out by Smolen and Weigand,\(^7\) use of the “estimated plasma concentration” is advantageous especially in the following cases; (1) only a non specific assay method which does not distinguish between intact drug and metabolite is available, (2) active metabolites play an important role in the pharmacologic activity of a drug and (3) the total (bound plus unbound) concentration of intact drug is not directly related to the biophase drug concentration and the pharmacologic response. In such cases, the pharmacokinetic data obtained by direct chemical or radiological assay will not correlate well with the pharmacologic response. This is the reason why we used the “estimated plasma concentration,” instead of actual plasma concentration of captoril, in this study.

In the previous study, we also determined plasma concentrations of captoril after i.v. ad-
administration both in NR and SDR, using an HPLC method, and found that the actual values and estimated values for captopril plasma concentration were almost identical with one another. Consequently, estimated plasma concentrations of captopril in GHR presumably represent the actual plasma concentrations as well.

In the present model, the hypotensive or antihypertensive effect of captopril was assumed to be solely attributable to the reduction of Ang II level in the body, and no feedback mechanism for the regulation of blood pressure was incorporated into the model. The overshooting phenomenon observed in SDR and GHR at the recovery phase of MAP is a typical characteristic of feedback mechanisms of blood pressure regulation. These results may indicate that an auto-regulation system of blood pressure, including feedback mechanisms, should be incorporated into the model, especially in the disease state.

As shown in the results section, the predicted values for MAP at the recovery phase in both SDR and GHR did not match the experimental results very well. There might be several reasons for this discrepancy; (1) the pharmacokinetic properties of exogenous angiotensins were different from those of endogenous angiotensins, (2) the time course of the plasma ACE activity after captopril administration does not necessarily reflect that of the total ACE activity in the body, and (3) the non-angiotensin (angiotensin-independent) mechanisms also play an important role in the action of captopril. None of these possibilities can be ruled out at present; however, the last one is the most likely reason for the discrepancy. As discussed in the preceding paper, the kallikrein–kinin system is known to be important in low-renin (and therefore low-angiotensin) hypertension. Since we did not determine the concentrations of kinins in the present study, the role of the kinins in captopril action in high-renin rats is still unclear. Further investigation is required in this respect.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research (58460229) from the Ministry of Education, Science and Culture, Japan.

References