Plasma Concentration and Acetylator Phenotype Determine Response to Oral Hydralazine

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SUMMARY The vasodepressor response to single and multiple oral doses of hydralazine, 1 mg/kg, was studied in hypertensive patients. The concentration of hydralazine in plasma was measured both by a newly developed specific and a nonspecific assay similar to those used in previous studies. Acetylator phenotype was determined following oral sulfamethazine. Plasma hydralazine concentration peaked at 1 hour after administration and was undetectable 2 hours later. Apparent hydralazine was present in plasma in higher concentration and for a longer duration than hydralazine. The peak decreases in blood pressure (BP) were proportional to plasma hydralazine concentration following administration of both single and multiple doses and were substantially maintained for 8 hours. In contrast there was no significant correlation between decreases in BP and apparent hydralazine concentrations. The plasma concentration of hydralazine after a standard oral dose varied by as much as 15-fold among individuals and was lower in rapid than slow acetylator phenotype patients. The BP responses were positively correlated with plasma hydralazine concentrations and inversely correlated with acetylator indices. Low plasma concentrations may account for poor responses of some patients to conventional oral doses of hydralazine. The applicability of acetylator phenotyping for individualization of hydralazine dosage regimens merits further evaluation. (Hypertension 3: 580–585, 1981)

KEY WORDS • blood pressure • acetylator phenotype • dose response • hydralazine • plasma concentration

HYDRALAZINE is a drug of major importance in the treatment of hypertension. When used in combination with a diuretic and beta blocker, it has been shown to be effective in patients resistant to prior therapy. However, examples of inadequate blood pressure (BP) responses to regimens that include hydralazine are not infrequent. It would be clinically useful to have a systematic method by which patient responsiveness to hydralazine might be predicted and dosing regimens optimized for individual patients. At present, empiric guidelines specify a maximum dose of 200 mg/day, except for fast acetylators, for whom a maximum dose of 300 mg/day may be used. The larger recommended dose in fast acetylators is based on the lower concentrations of plasma hydralazine in fast acetylators relative to slow acetylators at equivalent oral doses and the infrequent occurrence of disseminated systemic lupus erythematosus (DSLE) in patients of fast acetylator phenotype. However, there are reasons to reexamine the status of current knowledge relating BP response to plasma hydralazine concentration.

At present, empiric guidelines specify a maximum dose of 200 mg/day, except for fast acetylators, for whom a maximum dose of 300 mg/day may be used. The larger recommended dose in fast acetylators is based on the lower concentrations of plasma hydralazine in fast acetylators relative to slow acetylators at equivalent oral doses and the infrequent occurrence of disseminated systemic lupus erythematosus (DSLE) in patients of fast acetylator phenotype. However, there are reasons to reexamine the status of current knowledge relating BP response to plasma hydralazine concentration.

Most compelling is recognition that the analytic methods used in prior studies are nonspecific, measuring a combination of hydralazine and acid labile metabolites for which the term "apparent hydralazine" has been suggested. The most abundant component of apparent hydralazine is the hydralazine pyruvic acid hydrazone (HPH), which does not reduce BP in animals. In addition, there is need for a more quantitative estimate than is now available of the extent to which interindividual differences in plasma hydralazine concentration may account for differences in BP response.

We have established a positive correlation between the antihypertensive effect and the plasma concentration of hydralazine measured by a specific method. There was no correlation between antihypertensive effect and plasma apparent hydralazine measured by a nonspecific method. In addition, we have shown that acetylator phenotype is inversely related to hypoten-
sive response following oral administration of a standard (1 mg/kg) dose of hydralazine. The mechanism by which the acetylator index determines the response to hydralazine is probably related to its influence on hydralazine bioavailability and thereby on hydralazine plasma concentration. Since the acetylator phenotype is easy to determine, it has a marked advantage over the determination of plasma levels for evaluation of cardiovascular responses to hydralazine.

Methods
Nine hypertensive male patients who required hydralazine for clinical management of essential hypertension were admitted to the Special Diagnostic and Treatment Unit at the Audie Murphy Veterans Hospital (table 1). The patients had no clinical evidence of angina pectoris, prior myocardial infarction, or cerebral vascular accident. Six days before the study, all medications were discontinued except those noted in table 1. Upon admission, 4 days were allowed for acclimatization to the hospital environment. Diet in the hospital contained 8 g of salt/day. Electrolyte balance was not quantitated. There was no consistent trend of body weight throughout the hospital stay.

Each patient underwent pharmacodynamic and pharmacokinetic studies following hydralazine administration on three occasions. The three studies were: single oral dose; single intravenous dose; and multiple (fifth) oral dose. The multiple dose study was conducted last in each patient due to the known persistence of BP effects after repeated doses of hydralazine. On the fourth and eighth hospital days, patients were given either a single oral dose of hydralazine, 1 mg/kg, or a single intravenous bolus of hydralazine, 0.3 mg/kg. Following the second study, the patients were started on a maintenance oral dose of hydralazine, 1 mg/kg, 9:00 am and 9:00 pm. A combination of commercial Apresoline tablet sizes was used to approximate the maintenance dose. On the morning of the fifth oral dose, we repeated the study of plasma concentration and BP effects (third study). The results of the i.v. study are reported in a separate manuscript.

On study days, patients had nothing by mouth except water beginning the prior midnight. The patients remained recumbent in bed without smoking throughout the study. At 7:00 am, a pediatric scalp vein needle was placed in a forearm vein for blood sampling. The BP was measured in the arm at 10-minute intervals by an Arteriosonde. A solution of 5% dextrose and water was slowly infused through a second intravenous needle in the other arm used for intravenous hydralazine administration. At 9:00 am hydralazine was administered, either orally or intravenously over a 100-second interval. The oral hydralazine dose solution was made by mixing an appropriate volume of hydralazine for intravenous injection with 30 ml of distilled water immediately before administration. Blood was sampled every 5 minutes × 12, every 15 minutes × 8, and every 30 minutes until 8 hours had elapsed. A light lunch was provided at 1 pm.

Acetylator index was determined during the equilibration period by administration of an oral 10 mg/kg dose of sulfamethazine (Matheson, Coleman and Bell, Norwood, Ohio). Acetylated and total sulfamethazine concentrations were measured in a plasma sample taken 6 hours later. The 6-hour plasma ratio of acetylated-to-total sulfamethazine has been shown optimal for purposes of phenotyping.

The handling of blood specimens for hydralazine analysis, which has been described elsewhere, was performed in the minimum possible time. Analysis for hydralazine and apparent hydralazine was performed by published methodology. The lower limits of detection for the two assays were 0.0125 and 0.1 μM respectively.

Analysis of Data
Acetylator index was defined as the ratio, multiplied by 100, of acetylated-to-total sulfamethazine in plasma. Peak plasma hydralazine and apparent hydralazine concentrations were taken as the highest determined values. The area under the curve (AUC) for hydralazine and for apparent hydralazine was calculated by the trapezoid method, extrapolated to infinity. The terminal t½ of fast acetylator patients was assumed to be the slope of the third and final phase of decay from plasma after the intravenous dose administered on a separate occasion. This provided a maximum estimate of AUC after oral administration. All BP values were expressed as mean arterial pressure (MAP), which was calculated as diastolic pressure plus 1/3 pulse pressure.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Endogenous creatinine clearance (ml/kg/min)</th>
<th>Concomitant drug therapy</th>
<th>Acetylator index*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>75</td>
<td>39</td>
<td>Hct, digoxin, chlorproparamide</td>
<td>36</td>
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<tr>
<td>2</td>
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<td>100</td>
<td>92</td>
<td>Furosemide, digoxin, chlorproparamide</td>
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<td>53</td>
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<td>73</td>
<td>Hct</td>
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<td>9</td>
<td>47</td>
<td>116</td>
<td>120</td>
<td>Hct, allopurinol</td>
<td>70</td>
</tr>
</tbody>
</table>

*Acetylator index = 500 × [acetylated sulfamethazine/total sulfamethazine] in plasma 6 hours after 10 mg/kg sulfamethazine administered orally.

Hct = hydrochlorothiazide.
During each of the three studies, BP was measured 12 times at 10-minute intervals prior to administration of hydralazine. Analysis of variance indicated that the predrug BPs of the first study were not significantly different from those of the second study, i.e., (mean ± SE, mm Hg) 120 ± 1 and 118 ± 1 mm Hg respectively. Since a persistent hypotensive effect of the previous hydralazine dose was expected at the time of the third study, predrug pressures from the single oral study were designated as baseline for calculation of drug effect following both the single and fifth oral dose studies. Analysis of variance indicated that no significant variation in MAP occurred for 2 hours prior to hydralazine administration on each of the three study occasions. The MAP subsequent to dose administration was calculated as the moving average of three pressures, one before and one after the designated time. The maximum change in MAP (Δ MAP) from baseline was chosen for analysis of drug effect.

Statistical analysis was performed by methods specified in the text. The methods included: Student's t for paired and unpaired data, Duncan's new multiple range test, and multiple least squares stepwise linear regression. Two-way analysis of variance was performed with the University of Texas Health Science Center at San Antonio computer facility, using the 1977 version of BMDP 2V program.

Results

Plasma Concentration of Hydralazine and Apparent Hydralazine

Plasma concentrations of hydralazine peaked 10 to 30 minutes after administration of either a single oral dose or the fifth oral dose. Plasma concentrations then decayed rapidly, becoming undetectable 60 to 180 minutes after administration. The average range of peak plasma concentrations of hydralazine and apparent hydralazine following the single and fifth doses are presented in tables 2 and 3. Concentrations of hydralazine following the single and fifth dose were not significantly different. The concentration of apparent hydralazine rose to a peak which was 11.6 ± 3.5 times that of hydralazine after the single dose, and 25.2 ± 8.8 times that of hydralazine after the fifth dose. The increase in this ratio indicated accumulation of apparent hydralazine with repetitive dosing. Accumulation was consistent with a significant residual concentration of apparent hydralazine (1.49 ± 0.37 μM, t test) in plasma prior to administration of the fifth dose of hydralazine. The average area under the plasma concentration time curves for hydralazine (AUCH) after the single and the fifth oral doses were strikingly small relative to the AUCs of apparent hydralazine (AUCAH). The average ratio of AUCH/AUCAH following the single oral doses was 0.014 ± 0.0032. The ratio was significantly (p < 0.02, paired t test) smaller after multiple oral doses.

TABLE 2. Mean and Range of Peak Plasma Concentrations of Hydralazine ([H]p) and Areas under the Plasma Concentration-Time Curve of Hydralazine (AUCH) after the Single and Fifth Oral Doses of Hydralazine (1 mg/kg) in Nine Hypertensive Men

<table>
<thead>
<tr>
<th>Dose</th>
<th>[H]p μM</th>
<th>AUCH μM min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>sequence mean</td>
<td>SE</td>
</tr>
<tr>
<td>Single</td>
<td>0.62</td>
<td>0.23</td>
</tr>
<tr>
<td>Fifth</td>
<td>0.51</td>
<td>0.17</td>
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</tbody>
</table>

Blood Pressure Responses to Hydralazine: Plasma Levels

The average values of MAP for 2 hours preceding and 8 hours after oral hydralazine administration are illustrated in figure 1. The individual peak reductions in MAP occurred from 30 to 140 minutes after the first oral doses and 60 to 180 minutes after the fifth oral doses. Data for the group as a whole show there was strong evidence of a persistent effect 10 to 12 hours following the fourth dose in the multiple oral dose sequence. The level of baseline MAP was significantly (p < 0.001) less preceding the fifth dose than preceding the first dose (two-way analysis of variance).

While the predose MAP was significantly less prior to the fifth dose than prior to the single dose, the absolute pressure at peak effect after the fifth dose was equal to that at peak effect after the first dose; i.e., there was no cumulation of peak effect on BP. In addition, recovery of MAP during the 8 hours after administration of either the single or fifth dose was not significant (Duncan's new multiple range test).

There was a significant correlation between reductions in MAP from baseline and plasma hydralazine concentration following both the single and fifth oral dose (least squares regression).

These relationships are illustrated in figure 2 for both peak hydralazine concentration and AUCH. The ranges from lowest to highest values for peak hydralazine plasma concentrations and AUCH were

*This program was developed at the Health Science Center computing facility, UCLA, under sponsorship of NIH Special Research Resources Grant RR-3 from the National Institutes of Health.
FIGURE 1. Time course of mean arterial pressure (MAP) for 2 hours preceding and 8 hours following a single dose and the fifth of multiple oral doses of hydralazine, administered at 12-hour intervals. All oral doses were 1 mg/kg body weight. Results presented as mean ± SE.

FIGURE 2. Relationships between peak reduction in mean arterial pressure (MAP) and plasma concentrations of hydralazine following a single dose (left panels) and the fifth dose (right panels) of hydralazine, 1 mg/kg. Hydralazine concentrations are expressed as both peak plasma concentration (upper panels) and area under the plasma concentration-time curves, AUCH (lower panels).

FIGURE 3. Relationships between peak reduction in mean arterial pressure (ΔMAP) and acetylator index (AI). following a single dose (left) and the fifth dose (right) of hydralazine, 1 mg/kg orally.
approximately 15-fold. The effect/dose slopes did not differ significantly among the four relationships. There were no significant correlations of ΔMAP with peak concentration, 2-hour concentration, or AUC of apparent hydralazine, following either the single or the fifth dose of hydralazine.

Blood Pressure Response to Hydralazine: Acetylator Phenotype

Among the nine subjects, the acetylator index ranged from 18 to 82 (table 1). The distribution of the acetylator index was not bimodal but rather included one or two patients in each decile from the second through the ninth. There were significant ($p < 0.05$) inverse relationships between ΔMAP and acetylator index following both the single and the fifth oral doses of hydralazine (figure 3).

Discussion

Our results establish a relationship between plasma concentration of hydralazine as determined by a specific assay and the magnitude of hypotensive effect after oral administration of hydralazine. This correlation was significant following both single dose and the fifth dose of a series administered at 12-hour intervals. These data imply that a major factor accounting for interindividual differences in the response to oral hydralazine is the plasma concentration of the unmetabolized drug. Other factors, in particular the absolute level of pretreatment BP, may also be expected to influence the magnitude of response (Shepherd et al., unpublished data). There was no evidence of accumulation of hydralazine in plasma following five doses. Likewise, the absolute BP at peak response was the same following the single and the fifth doses. Unlike Zacest and Koch-Weser,\textsuperscript{1} we could find no correlations between BP responses and the concentrations of apparent hydralazine. The plasma concentrations of apparent hydralazine as measured by Zacest and Koch-Weser would be in close agreement with those that we determined using the Jack method.\textsuperscript{21} We tested possible correlations of BP response with the 2-hour plasma concentration of apparent hydralazine as well as peak concentration and AUC, since blood sampling was performed 2 hours after dosing in previous studies which have demonstrated relationships between BP response and plasma apparent hydralazine concentrations.\textsuperscript{1, 15} Perhaps the correlation of effect with plasma apparent hydralazine improves with longer duration of administration, as in the studies conducted by Zacest and Koch-Weser\textsuperscript{1} and by Jounela et al.\textsuperscript{8}

Our negative observations are consistent with the knowledge that the Jack assay is nonspecific, measuring not only hydralazine but also acid labile hydralazine conjugates, including hydralazine pyruvic acid hydrazone (HPH), which has been shown to constitute the major proportion of the substances assayed by nonspecific methods.\textsuperscript{18, 14} Since HPH produces no hypotensive effect in the rabbit,\textsuperscript{19} rat (Clementi, unpublished data), and dog,\textsuperscript{18} it presumably does not contribute to the hypotensive effect of administered hydralazine in the human. Inclusion of a large and variable proportion of HPH in the nonspecific assay results is ample basis for the absence of a good correlation of plasma concentration with antihypertensive effect.

A principal factor accounting for interindividual differences in hydralazine concentration after a standard oral dose is acetylator phenotype. It was recognized by Zacest et al.,\textsuperscript{1} Reidenberg et al.,\textsuperscript{4} and Talseth\textsuperscript{16} that the concentrations of apparent hydralazine after oral administration were lower in fast acetylators than in slow acetylators. In addition, we have established an inverse correlation between acetylator index and both the peak plasma concentration of hydralazine and the AUC\textsubscript{0}.\textsuperscript{17} It seems most probable that the inverse relationship of acetylator index to BP response is due to the former's effect on bioavailability and therefore the plasma concentration of hydralazine at the doses we studied. There is the additional possibility that acetylator index influences the response to hydralazine through a pharmacodynamic mechanism in addition to the postulated pharmacokinetic mechanism (Shepherd et al., unpublished data).

The distribution of acetylator index among our patients was consistent with recent kinetic studies that have shown a trimodal, rather than bimodal, population distribution.\textsuperscript{22} On the basis of our results, we think that there is an advantage to expressing acetylation capacity as the absolute value of the acetylator index rather than dichotomously as slow and fast.

The data imply that, for patients with a high acetylator index, the dose of hydralazine required for a substantial vasodepressor response may be quite large, i.e., up to 15 times as large as the dose required for an equivalent effect in the slowest acetylators. Taking a 30 mm Hg vasodepressor response as an example, and the range of acetylator index observed in our group of patients, we estimate the doses would be 2 mg/kg/day for a patient with the lowest acetylator index and 30 mg/kg/day for a patient with the highest acetylator index. Since a dose of 4 mg/kg/day is the current upper limit of the recommended dosage range for hydralazine, the most rapid acetylators would be expected to develop approximately one third of the desired responses to hydralazine. This must be considered a possible factor in the poor response to hydralazine seen in some patients.\textsuperscript{2, 8} That plasma concentration of hydralazine may not be the only factor in refractoriness to hydralazine is suggested by the failure of some patients who were designated as "slow" acetylators to respond to daily doses of 200 to 400 mg.\textsuperscript{28} We propose that it is necessary to measure the plasma hydralazine concentration to conclusively establish clinical refractoriness to hydralazine. Alternatively, measurement of acetylator index may provide a clinically adequate estimate of plasma hydralazine concentration relative to oral dose. In addition, compliance must be unequivocally established.
We conclude that patients with a high acetylator index need a greater than 50% increment in the now recommended maximum daily dose of 200 mg if they are to have plasma concentrations of hydralazine that would produce a substantial antihypertensive effect. The practical usefulness of acetylator index in predicting the dose of hydralazine required to produce a desired vasodepressor response remains to be established. If acetylator index should provide a guideline of this type, potentially unresponsive patients could be spared the time and expense of an unsuccessful therapeutic trial of hydralazine. Alternatively, doses larger than 300 mg/day might be given patients with a high acetylator index if the toxicity of hydralazine, like its efficacy, is related to its plasma concentration.

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Plasma concentration and acetylator phenotype determine response to oral hydralazine.  
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doi: 10.1161/01.HYP.3.5.580  
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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