[Chem. Pharm. Bull. 31(1) 247—255 (1983)]

Hydralazine–Phenobarbital Interactions in Rats. II. Disposition of Hydralazine and Its Acid-labile Conjugates, and Liver Drug-metabolizing Enzyme Activities in Normotensive and spontaneously Hypertensive Rats

TARO OGISO,* MASAHIRO IWAKI and NOBUMICHI OHTSUKI

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3-4-1, Higashi-Osaka 577, Japan

(Received June 24, 1982)

The effects of intraperitoneal treatment with hydralazine hydrochloride 5 mg/kg and phenobarbital 2.5 mg/kg daily for 7 d on the elimination of hydralazine (HP) and its acid-labile conjugates (ALC), on liver enzyme activities, and on hepatic and renal functions in both normotensive and spontaneously hypertensive rats (SHR) were studied. The rate constant (0.445 ± 0.078 h^{-1}) for the elimination of HP from plasma in normotensive rats was significantly higher after the repeated treatment than that (0.205 ± 0.085 h^{-1}) found in a single dosing study, while the rate constant after repeated treatment in SHR was only slightly higher ($k = 0.371 \pm 0.058$ and 0.273 ± 0.059 h^{-1} for repeated and single administrations, respectively). The elimination of ALC from plasma was also enhanced by the repeated administration in both types of rats. Comparative *i.v.* injection of the combined drugs showed that the plasma HP concentration–time curve was biphasic and the terminal elimination rate constant of HP was almost the same as that for a single *i.p.* injection. The repeated dosing of the combined drugs significantly enhanced the drug-metabolizing enzyme activities in microsomes as compared with those of the control, but did not change the *N*-acetyltransferase activity of the liver. In normotensive rats, the repeated treatment of the combined drugs led to a significant increase in hepatic and renal clearance, whereas in SHR the same treatment had little effect. These results suggest that the difference in the plasma elimination of HP between normotensive rats and SHR was mainly due to the different extents of enhancement of hepatic and renal clearance following the repeated treatment.

Keywords——interaction of hydralazine and phenobarbital; disposition of hydralazine and its acid-labile conjugate; drug-metabolizing enzyme activity; hepatic and renal clearance; hydralazine

The coadministration of two or more drugs has been widely used for the therapy of hypertension; many hypotensors are frequently used with β-adrenergic blocking agents and sedatives. The era when phenobarbital (PB) was virtually the only drug recommended for sedation has passed, but the coadministration of hydralazine (HP) and barbiturates is still used clinically. We have previously reported[1] a clear-cut correlation between the pharmacological effect and the plasma level of HP, and showed that the hypotensive effect in normotensive rats is significantly decreased after the repeated administration of HP and PB in combination as compared with that following a single dose, while the decrease in the effect in spontaneously hypertensive rats (SHR) is less marked than that in normotensive rats. Such drug interactions may lead to ineffective therapy in the treatment of hypertensive patients. Therefore, it is necessary to elucidate the characteristics of the drug interaction of HP and PB.

In this study, in an attempt to clarify the mechanisms of the decrease in hypotensive effect after repeated treatment and of the difference in the decrease between normotensive and hypertensive rats, the disposition of HP and its acid-labile conjugates (ALC), the liver drug-metabolizing enzyme activities, and the hepatic and renal clearance in both types of rats following 7 d' treatment with HP and PB were determined in comparison with those in a single dosing group.

Experimental

Materials——Hydralazine hydrochloride and 1-hydrazino-4-methylphthalazine, an internal standard for gas-liquid chromatography, were obtained from Ciba-Geigy Co., Ltd. Phenobarbital was purchased from Hoei Yakko Co., Ltd. Nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate (G-6-P) dehydrogenase and G-6-P were obtained from Oriental Yeast Co., Ltd. Acetyl Coenzyme A (lithium salt) was purchased from Sigma Chemical Co. Aniline, used as substrate for the enzyme reaction, was redistilled. All other chemicals used were of the highest grade commercially available.

Animals and Treatment——Male normotensive Wistar rats weighing 90—100 g and male Okamoto spontaneously hypertensive rats (SHR) weighing 250—350 g were used throughout. The animals were divided at random into 3 groups (control, single dose and repeated dose), each consisting of 4—10 rats. Animals were treated by the same procedure as described in a previous paper.[1]

Determination of HP and ALC in Plasma——Animals were fixed on the operating table without anesthesia. Blood specimens were collected in heparinized syringes from the right femoral vein and plasma was obtained immediately by gentle centrifugation. The plasma concentrations of HP and acid-labile conjugates (ALC) were determined according to the method of Zak *et al.*[2] by GLC using a Hitachi 163 gas chromatograph with an electron capture detector and a 3 mm×1 m glass column packed with 3% OV-225 on Chromosorb W-HP (80—100 mesh). The amount of ALC was calculated by subtracting that of unchanged HP from the total HP. All analyses were carried out within 30 min of obtaining a blood sample.

Determination of Indocyanine Green and *p*-Aminohippuric Acid in Plasma——Animals were treated with a single or repeated administration of HP-PB or HP alone. Indocyanine green (ICG) (5 mg/kg) for hepatic clearance or *p*-aminohippuric acid (PAH) (30 mg/rat) for renal clearance (separately) was injected intravenously 2 h after the final dosing of the drugs. ICG and PAH in plasma were determined according to the methods of Shimizu *et al.*[3] and Brun *et al.*,[4] respectively.

Preparation of Rat Liver Microsomal and Soluble Fractions——Animals were starved for 24 h and sacrificed by decapitation 2 h after the final dosing of saline or the drugs. The liver was thoroughly perfused *in situ* with cold 0.9% NaCl solution through a portal vein. The liver microsomal and soluble fractions were prepared according to the method of Omura and Sato.[5] Both microsomal and soluble fractions were immediately chilled and used for the measurements within 24 h.

Assay of Drug-metabolizing Enzyme Activities——Aniline *p*-hydroxylase activity was measured according to the method of Ikeda.[6] Cytochrome P-450 was determined by the method of Omura and Sato.[5] *N*-Acetyltransferase activity in the soluble fraction was estimated with *p*-aminobenzoic acid as a substrate and by the method described by Hearse and Weber.[7]

Protein Determination——Protein concentration was estimated by the procedure described by Lowry *et al.*[8]

Pharmacokinetic and Statistical Analysis——The area (AUC) under the plasma drug concentration-time curve after administration was determined by use of the trapezoidal rule to 4.5 h after the injection and the area beyond the last observed plasma concentration (C_n) was added by integration (C_n/k), where k, the rate constant for elimination, was calculated from the equation, $k=0.693/t_{1/2}$, where $t_{1/2}$ is the elimination half-life. The logarithmic concentration of the drug or metabolite was plotted against time after administration. The pharmacokinetic constants were calculated from the linear part of the plots using the least-squares method. Plasma clearance was calculated by dividing the dose by the area under the plasma level-time curve. The apparent volume of distribution (V_d) was calculated from the ratio of the given dose to the plasma concentration (C_0) extrapolated to zero time. Hepatic clearance was estimated as the product of the elimination rate constant (k_{el}) and the apparent volume of distribution of ICG, and renal clearance was calculated by dividing the dose by the area under the plasma concentration-time curve of PAH. Renal plasma flow rate (RPF) was calculated according to the following equation:[9] $RPF=V\cdot\ln 2/t_{1/2}$, where V is the apparent volume of PAH distribution.

The data were compared by an analysis of variance. Statistical evaluations were performed with Student's t-test, with $p<0.05$ as the criterion of significance. Correlation analyses were carried out by the least-squares regression method.

Results

Plasma Concentrations of Hydralazine and Acid-labile Conjugates following Single or Repeated Administration of the Combined Drugs in Normotensive Rats and SHR

Rats received a single or 7 d' treatment with the combined drugs (5 mg of HP and 2.5 mg of PB/kg of body weight, *i.p.*), and plasma concentrations of HP and ALC were measured following the final administration of the combined drugs. The results are shown in Fig. 1 (normotensive rats) and Fig. 2 (SHR). HP was rapidly absorbed and the peak plasma drug

concentration was reached within 0.75 h after *i.p.* injection. HP was eliminated from the plasma according to first-order kinetics in each group.

In normotensive rats, the elimination rate constant of 0.446 ± 0.078 h^{-1} for HP in the repeated dose group was significantly higher than that in the single dose group (0.205 ± 0.085 h^{-1}). ALC produced appeared in the plasma immediately after the absorption of HP and decayed according to a single first-order function. After the repeated treatment, the elimination rate of ALC was also increased ($k=0.142$ h^{-1} for the single dose group and $k=0.383$ h^{-1} for the repeated dose group).

In SHR, there was a general trend in the repeated dose group to show a slight increase in the elimination rate constant of HP as compared with a single dosing, but the effect was not statistically significant. The disposition of ALC from plasma was enhanced by the repeated treatment, as in normotensive rats. Some pharmacokinetic parameters of HP and ALC following a single or repeated *i.p.* injection are shown in Table I. The repeated treatment with the combined drugs in normotensive rats induced a significant decrease in the $AUC_{0-\infty}$ and a drastic increase in HP clearance, probably due to the enhanced elimination rate of HP. In SHR, however, the fluctuations of the parameters following the repeated treatment were not as marked as in normotensive rats.

Plasma Concentrations of Hydralazine and Its Acid-labile Conjugates after a Single *i.v.* Injection of the Combined Drugs in Normotensive Rats

The concentrations of HP and ALC in plasma following a rapid *i.v.* injection of the combined drugs are shown in Fig. 3. The plasma HP concentration declined in a biexponential manner and the terminal elimination rate constant (β) of HP was 0.199 ± 0.039 h^{-1}, which was almost the same as that in the single *i.p.* dose group. ALC levels in plasma were about 45 and 85 % of HP levels at 0.25 and 0.75 h after *i.v.* injection, respectively. These results suggest that the formation of ALC containing hydralazine pyruvic acid hydrazone is very rapid in the systemic circulation as a consequence of the reaction with endogenous carbonyl compounds and the drug.[2,10] The ALC in plasma beyond 0.75 h after the injection decayed in a first-order manner ($k=0.219$ h^{-1}). The value was different from that in the single *i.p.* dose group ($k=0.142$ h^{-1}).

Effect of Single or Repeated Administration of the Combined Drugs on Liver Drug-metabolizing Enzyme Activities and Liver Weight in Normotensive Rats and SHR

To clarify the mechanism of the enhanced plasma clearance of HP ater 7 d' treatment with the combined drugs, the liver drug-metabolizing enzyme activities and liver weights of rats after drug administration were estimated. As shown in Table II, repeated dosing of the com-

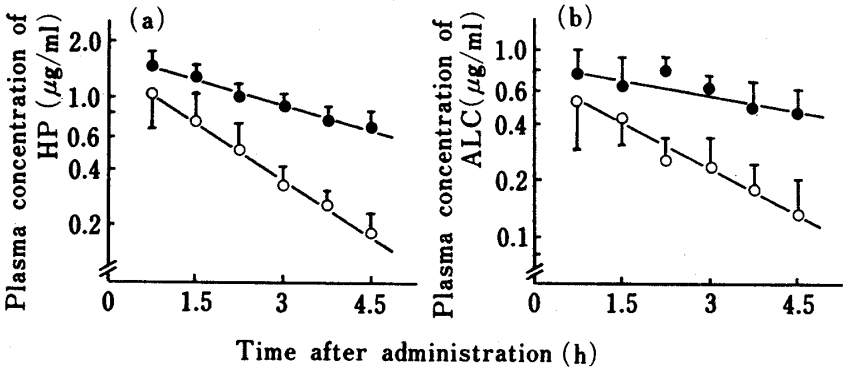

Fig. 1. Semilogarithmic Plots of Plasma Concentrations of Hydralazine and Acid-labile Conjugates after Single and Repeated Administrations of the Combined Drugs in Normotensive Rats

(a) HP (b) ALC expressed as HP. Each point represents the mean ±S.D. of 5 rats. ●, single dose group; ○, repeated dose group.

bined drugs enhanced aniline hydroxylase activity by about 64% in normotensive rats and by 35% in SHR, and cyt. P-450 content by 47% in both types of rats as compared with those in the control groups. The rats of the single dose group which were killed 2 h after administration had almost the same activities as the control. The activity of *N*-acetyltransferase in liver soluble fraction catalyzing the acetylation of HP was also estimated, but there was no significant difference in *N*-acetyltansferase activities between the single and repeated dose groups in the two types of rats. These findings suggest that the induction of liver microsomal drug-metabolizing enzymes after repeated treatment with the combined drugs is associated, at least

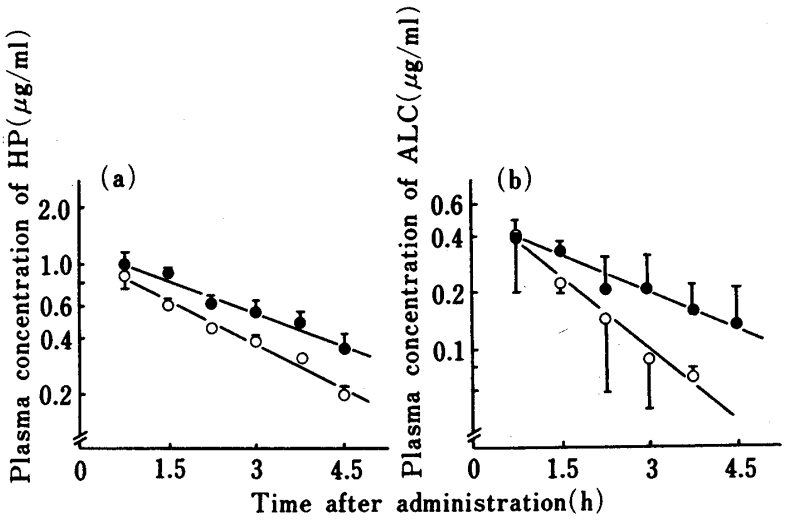

Fig. 2. Semilogarithmic Plots of Plasma Concentrations of Hydralazine and Acid-labile Conjugates after Single and Repeated Administrations of the Combined Drugs in SHR

(a) HP (b) ALC expressed as HP. Each point represents the mean ±S.D. of 4 rats. ●, single dose group; ○, repeated dose group.

TABLE I. Pharmacokinetic Parameters of HP and ALC after Single and Repeated Administrations (*i.p.*) of the Combined Drugs in Normotensive and Hypertensive Rats

Compounds	Parameters	Treatment	Normotensive rats	SHR
HP[a]	k (h^{-1})	Single	0.205±0.085	0.273±0.059
		Repeated	0.446±0.078[c]	0.371±0.058
	$t_{1/2}$ (h)	Single	4.01 ±1.99	2.64 ±0.63
		Repeated	1.60 ±0.33[d]	1.90 ±0.26
	V_d (l/kg)	Single	3.18 ±0.92	4.21 ±0.57
		Repeated	3.89 ±1.13	4.68 ±0.61
	AUC ($\mu g \cdot ml^{-1} \cdot h$)	Single	7.81 ±1.80	2.83 ±0.23
		Repeated	2.63 ±0.69[c]	2.03 ±0.09[c]
	Cl ($l \cdot h^{-1} \cdot kg^{-1}$)	Single	0.672±0.175	1.785±0.057
		Repeated	1.986±0.417[c]	2.362±0.087[c]
ALC[b]	k (h^{-1})	Single	0.142	0.283
		Repeated	0.383	0.588
	AUC ($\mu g \cdot ml^{-1} \cdot h$)	Single	5.76	1.534
		Repeated	1.58	0.822

a) Each value represents the mean±S.D. of 4—5 rats.
b) Values are obtained from the mean plasma concentration of 4—5 rats at each time.
c) $p<0.005$ in single *vs.* repeated dose groups.
d) $p<0.01$ in single *vs.* repeated dose groups.

partially, with the decrease in the hypotensive effect, probably due to an increase in HP clearance, while the acetylation of HP may contribute little to the observed enhancement of HP disposition. However, it is noteworthy that no significant difference was found in the induction of liver microsomal drug-metabolizing enzymes between normotensive and hypertensive rats, in spite of the higher HP clearance in normotensive rats than in SHR. No significant difference in relative liver weight was seen between the groups of normotensive rats and SHR.

In order to clarify the contribution of PB to the induction of the liver drug-metabolizing enzymes, the activities in the rats given HP alone were estimated. Although the repeated dosing of HP alone induced a slight enhancement (28 %) of *N*-acetyltransferase activity, no induction of other drug-metabolizing enzymes was seen after repeated treatment. Consequently, it seems that the stimulation of these enzyme activities after the combined drugs is mainly due to PB coadministered with HP.

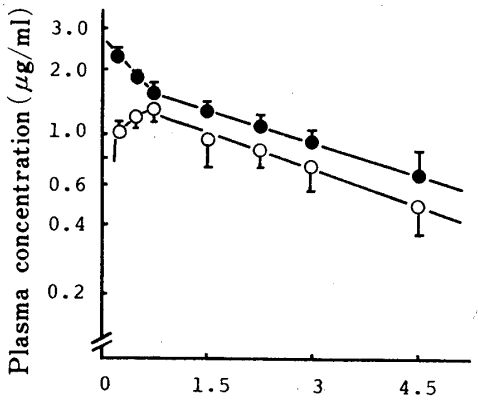

Fig. 3. Plasma Concentrations of Hydralazine and Acid-labile Conjugates after Single Intravenous Administration of the Combined Drugs in Normotensive Rats

Each point represents the mean ±S.D. of 4 rats. ●, HP; ○, ALC.

Indocyanine Green (ICG) Hepatic Clearance and *p*-Aminohippuric Acid (PAH) Renal Clearance after Single or Repeated Administration of the Combined Drugs in Normotensive Rats and SHR

Hepatic and renal clearance in normotensive rats and SHR, which had been pretreated

TABLE II. Effect of Single and Repeated Administrations of the Combined Drugs on Drug-metabolizing Enzyme Activities and Liver Weight

Enzymes	Treatment	Normotensive Rats		SHR	
		Activities	(%)	Activities	(%)
Relative liver weight (g/100 g of body weight)	Control	4.25 ±0.34	100	3.49 ±0.14	100
	Single	4.18 ±0.29	105	3.34 ±0.10	96
	Repeated	4.51 ±0.42	118	3.70 ±0.09[a,b]	105
Aniline hydroxylase*)	Control	0.489±0.031	100	0.445±0.017	100
	Single	0.541±0.068	111	0.419±0.020	92
	Repeated	0.800±0.096[c,d]	164	0.614±0.041[c,d]	135
Cytochrome P-450**)	Control	0.720±0.058	100	0.728±0.030	100
	Single	0.668±0.096	93	0.750±0.066	103
	Repeated	1.055±0.073[c,d]	147	1.072±0.118[c,e]	147
N-Acetyltransferase***)	Control	1.114±0.133	100	1.191±0.163	100
	Single	1.105±0.195	99	1.367±0.034	115
	Repeated	0.972±0.224	87	1.155±0.069[d]	97

Each value represents the mean±S.D. of 6—10 rats (in normotensive rats) or 4—6 rats (in SHR).
*) The enzyme activity is expressed as nmol of product per min per mg of protein.
**) The content of cytochrome P-450 is expressed as nmol per mg of protein.
***) The enzyme activity is expressed as nmol of acetylated *p*-aminobenzoic acid per min per mg of protein.

a) $p<0.05$ in control *vs.* repeated dose groups.
b) $p<0.025$ in single *vs.* repeated dose groups.
c) $p<0.005$ in control *vs.* repeated dose groups.
d) $p<0.005$ in single *vs.* repeated dose groups.
e) $p<0.01$ in single *vs.* repeated dose groups.

with a single or 7 d' dosing of the combined drugs, were estimated by using ICG and PAH. The plasma decline curves of ICG and PAH are shown in Fig. 4 (ICG clearance) and Fig. 5 (PAH clearance). In normotensive rats, the repeated treatment with HP and PB led to significant enhancements of the clearance ($Cl=8.38\pm0.68$ and 11.13 ± 1.47 ml·min^{-1}·kg^{-1} for the single and repeated dose groups, respectively, $p<0.05$) and elimination rate constant of ICG ($k_{el}=0.181\pm0.014$ and 0.220 ± 0.035 min^{-1} for the single and repeated dose groups, respectively, $p<0.01$). There was also a drastic increase in PAH elimination up to 60 min after the injection ($k_{el}=0.0399\pm0.0051$ and 0.0502 ± 0.0043 min^{-1} for the single and repeated dose groups, respectively, $p<0.01$) in the repeated dose group in comparison with that in a single dosing. RPF values of both groups are shown in Table III, and indicate that the renal plasma flow rate after repeated dosing was significantly enhanced as compared with that after a single dosing.

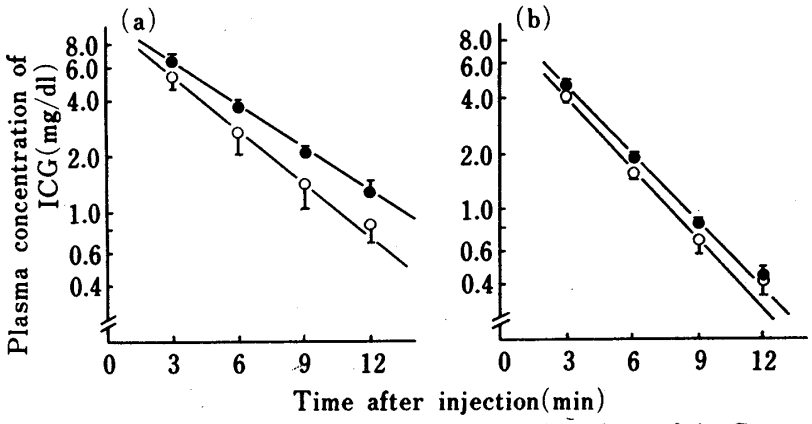

Fig. 4. Effect of Single and Repeated Administrations of the Combined Drugs on Hepatic Clearance of Indocyanine Green in Normotensive and Hypertensive Rats

Indocyanine green (5 mg/kg) was injected 2 h after administration of the drugs. Each point represents the mean ±S.D. of 4 rats. (a) Normotensive rats, (b) SHR. ●, single dose group; ○, repeated dose group.

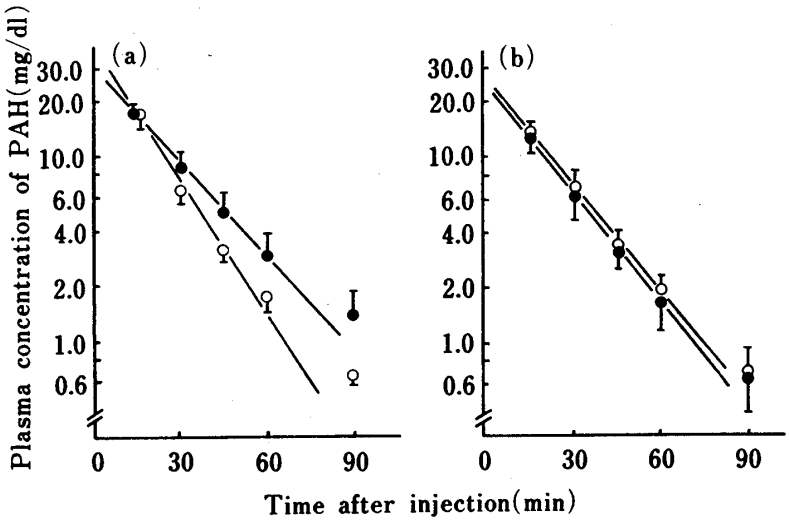

Fig. 5. Effect of Single and Repeated Administrations of the Combined Drugs on Renal Clearance of *p*-Aminohippuric Acid in Normotensive and Hypertensive Rats

Thirty mg of *p*-aminohippuric acid was injected 2 h after administration of the drug. Each point represents the mean ±S.D. of 4 rats. (a) Normotensive rats, (b) SHR. ●, single dose group; ○, repeated dose group.

TABLE III. RPF Values after Single and Repeated Administrations of the Combined Drugs in Normotensive and Hypertensive Rats

Rats	Single dose	Repeated dose
Normotensive rats	13.47±1.45	17.19±1.99[a]
SHR	23.64±3.12	21.30±1.54

Each value represents the mean±S.D. of 4 rats and is expressed as ml/min/kg.
a) $p<0.05$ in single *vs.* repeated dose groups.

In SHR, however, ICG clearance was slightly increased in the repeated dose group (Cl=13.70±0.36 ml·min^{-1}·kg^{-1} for the single dose group and Cl=16.28±0.50 ml·min^{-1}·kg^{-1} for the repeated dose group, $p<0.005$) as compared with the single dose group, but the elimination rate constant of ICG was not increased (k_{el}=0.259±0.004 and k_{el}=0.252±0.012 min^{-1} for the single and repeated dose groups, respectively). PAH clearance (Cl=9.42±1.41 ml·min^{-1}·kg^{-1}) in SHR was little changed following the repeated treatment as compared with the single dose group (Cl=9.46±1.68 ml·min^{-1}·kg^{-1}). No significant difference in RPF was observed between both groups of SHR as shown in Table III.

On the other hand, when the animals received HP alone for 7 d, no difference in ICG and PAH clearance or RPF was seen between the single and repeated dose groups in normotensive rats.

Discussion

The peripheral vasodilator, hydralazine, in combination with other drugs is used extensively in the treatment of moderate to severe hypertension.[11] The joint use of HP and PB is also not exceptional. We have previously reported[1] that in rats PB shows an additive or potentiating effect on the hypotensive effect of HP following a single administration of the combined drugs (HP 5 mg and PB 2.5 mg/kg of body weight), while repeated treatment with the combined drugs significantly decreases the hypotensive effect, although the decrease in the effect in SHR is not as marked as that in normotensive rats.

The present study showed that the repeated administration of the combined drugs significantly enhanced the disappearance of HP and ALC from the plasma in normotensive rats, whereas the same treatment in SHR slightly enhanced their elimination (Figs. 1 and 2). The decay of ALC plasma concentration was found to be proportional to that of the parent drug, HP. The absorption of HP was very rapid and peak plasma levels were obtained within 0.75 h following *i.p.* injection of the combined drugs. This is similar to the finding that, when HP was administered orally, the peak concentration appeared within 15—60 min after administration in most cases.[12-14] The terminal half-life ($t_{1/2}$) of HP (4.01±1.99 h) after a single *i.p.* injection of the combined drugs was similar to that (3.5—4.5 h) found in man during single dose studies.[15] The fact that the $t_{1/2}$ of HP after a single *i.v.* injection was almost the same as that following a single *i.p.* dosing suggests that the $t_{1/2}$ of HP in plasma may be independent of the route of administration.

It is shown that one important metabolic pathway of HP is the formation of hydrazones.[16] Recently, a procedure which allows measurement of both HP and ALC in plasma has been developed,[2] and some papers have appeared on the pharmacokinetics of ALC.[13,14] In our experiments, ALC appeared in the plasma very rapidly following *i.v.* injection of the parent drug, and the terminal slopes of the ALC decay curves could be described by first-order kinetics. It is suggested that the transformation of HP to ALC occurs very easily and rapidly in the systemic circulation, probably due to the reaction of HP with pyruvate and acetone in plasma. Reece *et al.*[10] demonstrated the formation of the pyruvate hydrazones from HP in

fresh plasma containing endogeneous pyruvic acid. It is reported that in man the plasma level of ALC is higher than that of HP.[2,13] However, our data showed that in rats the plasma level of ALC was slightly lower than that of HP, in accord with the results obtained in rats by Zak *et al.*[2] Schneck *et al.*[14] found a higher concentration of ALC in rats and their result is inconsistent with our data. It is difficult to speculate on the reasons for the differences in ALC levels in rats on the basis of the present data.

There is usually a close relationship between a reduction of pharmacological effect and the induction of drug-metabolizing enzyme activities. Therefore, the liver drug-metabolizing enzyme activities of the rats after administration of the combined drugs were estimated. The microsomal drug-metabolizing enzyme activities were significantly enhanced after the repeated treatment in both normotensive and hypertensive rats (Table II). However, no significant difference in induction of drug-metabolizing enzymes between normotensive rats and SHR was found, in contrast to our expectation. These observations make it unlikely that the decreased hypotensive effect after repeated treatment is wholly due to the induction of drug-metabolizing enzymes by the drugs.

On the other hand, recent reports have shown that the formation of various triazolo derivatives after acetylation of HP is an important metabolic pathway in man[17] and rats.[18] Thus, *N*-acetylation may play a key role in the metabolism of HP. In this study, no significant difference in *N*-acetyltransferase activities between the single and repeated groups of the two types of rats was found (Table II), indicating that acetylation of HP is not induced by the repeated dosing of the combined drugs and that it may be not a major cause of the reduced hypotensive effect after the repeated treatment. This view is supported by the finding that there was no difference between fast and slow acetylators in the terminal elimination half-life of HP, and that *N*-acetylation probably did not govern the elimination rate of HP in the postdistributive phase.[12]

It would be reasonable to assume that the reduction of hypotensive effect after the repeated treatment with the combined drugs may be partly ascribed to factors other than the induction of hepatic drug-metabolizing enzymes. Thus, hepatic and renal functions of rats after administration of the combined drugs were explored by estimating ICG and PAH clearance. In normotensive rats, the plasma elimination rate and clearance of ICG were significantly increased, by 22 and 33% respectively, after the repeated treatment in comparison with those after a single dosing ($p<0.05$). Additionally, the elimination rate of PAH was increased by about 25% following the repeated treatment. In SHR, ICG clearance was slightly enhanced (a 19% increase), while PAH clearance was not enhanced by the same treatment. These results indicate that hepatic and renal blood flow may be significantly enhanced in normotensive rats, but are little affected in SHR after the repeated treatment. We conclude from our data that enhanced hepatic and renal blood flow leads to the increase in plasma HP clearance following the repeated administration of the combined drugs and that the significant difference in the extent of decrease of hypotensive effect between normotensive rats and SHR after repeated treatment is mainly, but not wholly, due to the different extents of enhancement of hepatic and renal clearance. Since the repeated administration of HP alone did not enhance ICG and PAH clearance, the increase in hepatic and renal blood flow may be mainly attributed to the PB coadministered. It has been recognized that the young of humans and other animals may be more sensitive to drugs than adults. The characteristic differences in the hepatic and renal functions between normotensive and hypertensive rats after repeated dosing may be partially related to a difference in the sensitivity of these organs of the two types of rats to PB in the combined drugs. Ohnhaus *et al.*[19] reported a significant increase in urine volume and PAH clearance in PB-treated rats (30 $mg \cdot kg^{-1} \cdot d^{-1}$, for 4 d) and these changes were attributed to an increase in renal plasma flow or activated tubular transport following PB administration. In addition, PB is known to increase liver blood flow as well as to induce drug-metabolizing enzyme activities in rats,[20,21] and this increase in hepatic

blood flow influences the rate of elimination of exogenous compounds.[22] These reports support our view that the decreased hypotensive response after repeated dosing is mainly attributable to the increased drug-metabolizing enzyme activities of liver and the enhanced hepatic and renal clearance. HP was shown to be accumulated in renal failure, as indicated by unusually high plasma concentrations and a prolonged elimination half-life of HP.[23,24] Talseth[25] described a close correlation between the glomerular filtration rate and the elimination half-life of HP. Data from these reports suggest that the disposition of HP from plasma may be largely dependent upon the renal excretion. Therefore, the significant difference in the elimination rate of PAH between normotensive and hypertensive rats may reasonably explain the difference in the plasma clearance of HP in the two types of rats after repeated treatment with the combined drugs.

In conclusion, 7 d' treatment with HP and PB significantly increased the elimination rate of HP and ALC from plasma in normotensive rats, but only slightly increased it in SHR. In normotensive rats, the drastic decrease in the hypotensive effect after the repeated treatment with the combined drugs appears to be attributable to the significant induction of drug-metabolizing enzymes and the appreciable increase in hepatic and renal clearance, with a concomitant increase in the elimination rate of HP from plasma. The relatively slight change in the elimination rate of HP in SHR as compared with that in normotensive rats may be due to the rather insignificant increase in hepatic and renal functions, despite the increased drug-metabolizing enzyme activities.

References and Notes

1) T. Ogiso, M. Iwaki and H. Matsuoka, *Chem. Pharm. Bull.*, **30**, 3711 (1982).
2) S.B. Zak, G. Lukas and T.G. Gilleran, *Drug Metab. Dispos.*, **5**, 116 (1977).
3) R. Shimizu, M. Ichimura, Y. Noda, Y. Hamada, T. Saeki and N. Tonami, *Rinshobyori*, **20**, 879 (1972).
4) C. Brun and B. Anger, *J. Lab. Clin. Med.*, **35**, 152 (1950).
5) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).
6) M. Ikeda, *J. Biochem.*(Tokyo), **55**, 231 (1964).
7) D.J. Hearse and W.W. Weber, *Biochem. J.*, **132**, 519 (1973).
8) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1950).
9) H. Abe, T. Furukawa, H. Morita and S. Fukui, *Rinsyobyori*, **5**, 189 (1957).
10) P.A. Reece, P.E. Stanley and R. Zacet, *J. Pharm. Sci.*, **67**, 1150 (1978).
11) J. Kock-Weser, *New Engl. J. Med.*, **295**, 320 (1976).
12) T. Talseth, *Clin. Pharmacol. Ther.*, **21**, 715 (1977).
13) D.D. Shen, J.P. Hosler, R.L. Schroder and D.L. Ararnoff, *J. Pharmacokinet. Biopharm.*, **8**, 53 (1980).
14) D.W. Schneck, J.S. Sprouse, K. Miller, J.E. Vary, F.O. DeWitt and A.H. Hayes, *Clin. Pharmacol. Ther.*, **24**, 714 (1978).
15) M.M. Reidenberg, D. Drayer, A.L. DeMarco and C.T. Bello, *Clin. Pharmacol. Ther.*, **14**, 970 (1973).
16) T. Talseth, *Europ. J. Clin. Pharmacol.*, **10**, 375 (1976).
17) H. Zimmer, R. Glaser and J. Kokosa, *J. Med. Chem.*, **18**, 1031 (1975).
18) K.D. Haegle, H.B. Skrdlant, N.W. Robie, D. Lalka and J.L. McNay, *J. Chromatography*, **126**, 517 (1976).
19) E.E. Ohnhaus and H. Siegel, *Arch. Int. Pharmacodyn. Ther.*, **223**, 107 (1976).
20) A.S. Nies, G.R. Wilkinson, B.D. Rush, J.T. Strother and D.G. McDevitt, *Biochem. Pharmacol.*, **25**, 1991 (1976).
21) M.S. Yates, C.R. Hiley, P.J. Roberts and F.E. Crawford, *Biochem. Pharmacol.*, **27**, 2617 (1978).
22) G.R. Willkinson and D.G. Shand, *Clin. Pharm. Ther.*, **18**, 377 (1975).
23) H.M. Perry, H.A. Schroeder and J.D. Morrow, *Am. J. Med. Sci.*, **228**, 405 (1954).
24) R. Zacet and J. Kock-Weser, *Clin. Pharmacol. Ther.*, **13**, 420 (1972).
25) T. Talseth, *Clin. Pharmacokin.*, **2**, 317 (1977).