ANTIHYPERTENSIVE AND ANTIDIURETIC EFFECTS OF
3-HYDRAZINO-6-[N, N-BIS (2-HYDROXYETHYL) AMINO]-
PYRIDAZINE (L 6150) IN RATS

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Abstract—3-Hydrazino-6-[N,N-bis (2-hydroxyethyl)amino]-pyridazine (L 6150) has
been reported as an antihypertensive vasodilator drug. We determined antihypertensive
effect of L 6150 for 11 weeks in spontaneously hypertensive (SHR) rats, deoxycorticos-
terone and salt (DOC) hypertension, and renal hypertension due to clipping (CLIP).
The effects of hydralazine (HZ) and ecarazine (EZ) were also determined for comparison.
L 6150, HZ, and EZ showed antihypertensive effects in SHR, DOC and CLIP hyper-
tensive rats. These drugs increased heart rate in SHR and DOC rats. In CLIP
hypertension heart rate tended to be higher for 9–10 weeks after the treatments. These
treatments diminished incidence of the vascular disease in DOC and CLIP. We also
determined renal effects of L 6150, HZ and EZ in normal rats. These drugs decreased
urine volume, and excretion of osmotically active solutes, Cl, Na, and K for 180 min
after bicarbonate saline load. It is concluded that L 6150 is an antihypertensive
drug with characteristics of the vasodilator in rats.

3-Hydrazino-6-[N,N-bis (2-hydroxyethyl)amino]-pyridazine (L 6150) is a hydrazino-
pyridazine derivative characterized by the presence of a tertiary amine group in position 6.
A series of these derivatives has been synthetized to search for hydralazine compounds free
from unfavorable effects during long term treatment with higher doses (1). L 6150 has
showed depressor and antihypertensive activities in rats, dogs, and cats mainly due to a direct
action on the peripheral blood vessels (2–4). The fall of peripheral vascular resistance was
accompanied by an increase in cardiac output and heart rate (4–6). L 6150 has been reported
to be better tolerated in dogs than hydralazine (4).

The antihypertensive effect has been studied only for a few days (4). The duration
was too short to draw a definite conclusion on its antihypertensive action, since the therapy
in humans usually lasts for years. Therefore, we have extended the observation for 11
weeks in hypertensive rats: spontaneous, deoxycorticosterone, and renal hypertension.
We also studied diuretic effect of L 6150, because the antihypertensive vasodilator drugs
retained sodium (7). The effects of hydralazine and ecarazine, widely used antihypertensive
vasodilator drugs, were also determined for comparison.

MATERIALS AND METHODS

Studies in hypertensive rats

Experimental design: A total of 15 experimental groups, 9–10 rats each, made by
various combinations of hypertensive rats and drugs was studied (Table 1). The drugs were administered orally by a gastric tube for 11 weeks, 5 days per week. Student's t-test was used for statistical analysis.

**Table 1. Experimental design**

| Hypertension | Drugs |
|--------------|-------|
| SHR          | SOL   |
| SHR          | L4    |
| SHR          | L12   |
| SHR          | HZ    |
| SHR          | EZ    |
| DOC          | SOL   |
| DOC          | L4    |
| DOC          | L12   |
| DOC          | HZ    |
| DOC          | EZ    |
| CLIP         | SOL   |
| CLIP         | L4    |
| CLIP         | L12   |
| CLIP         | HZ    |
| CLIP         | EZ    |

SHR: spontaneously hypertensive rat, DOC: deoxycorticosterone and salt hypertension, CLIP: renal hypertension due to unilateral clipping without contralateral nephrectomy, SOL: solvent, L4 and L12: L 6150 4 and 12 mg/kg per day, p.o., HZ: hydralazine 4 mg/kg per day, p.o., EZ: ecarazine 12 mg/kg per day, p.o.

**Hypertensive rats:** In female spontaneously hypertensive (SHR) rats of F29-30, weighing 180-200 g from the colony of the Department of Pharmacology, Jichi Medical School, the drug treatment was started at age of 13 weeks. Deoxycorticosterone and salt (DOC) hypertension was made in female rats of HOS®: Donryu strain weighing 180-210 g, unilaterally (left side) nephrectomized, giving 10 mg/ml NaCl solution as drinking fluid, and treating with deoxycorticosterone acetate 10 mg/kg per week s.c. Dose of the corticosteroid was decreased to 2.5-5 mg/kg when hypertension became critical 3-4 weeks after the treatment had begun. The drug treatment was started 6 weeks after the deoxycorticosterone injections had begun. Renal hypertension due to clipping (CLIP) was induced by constricting the left renal artery with a silver ribbon (slit width 0.2 mm) and leaving the contralateral kidney intact in female rats of HOS®: Donryu strain weighing 170-190 g. The drug treatment was started 6 weeks after the surgery.

**Determination of blood pressure and heart rate:** Tail blood pressure (BP) was determined by a rat tail manometer (Natume Seisakusho KN-0090) without anesthesia. The rat was warmed for ca. 1 min at 70°C. The apparatus detects blood flow pulses in the tail by a photoelectric sensor. The arterial blood flow was interrupted by applying pressure to the
tail through a pneumatic cuff. The blood flow reappeared when the cuff pressure was decreased. The value was approximately equal to the maximum blood pressure. Pulse rate of the tail artery was counted simultaneously, and referred as heart rate (HR). BP and HR were determined once a week during the week before and for 10 weeks after the drug treatment had started. The intervals between the drug administration and the determination were 6–9 hr, and randomized in each rat. At the end of the drug treatment, during the 11th week, mean BP was determined directly without anesthesia or restraint through a cannula inserted into the abdominal aorta a day before (8). An electronic BP recording system (YHP 1280C, YHP 8805A, and Yokogawa Electric 3046C) was used. Correlations between BP values determined indirectly at the tail during the 10th week and directly at the abdominal aorta were fairly good (r=0.679, 0.884, and 0.772, respectively, P<0.01, in SHR, DOC and CLIP rats). The indirect determination resulted in higher values after the drug treatment in SHR rats.

Drugs: 3-Hydrazino-6-[N,N-bis (2-hydroxyethyl)amino]-pyridazine 2HCl (L 6150) (Dow Lepetit Japan), hydralazine HCl (HZ) (Yamanouchi Pharmaceutical), and ecarazine HCl H2O (EZ) (Kyowa Hakko Kogyo) were used. The drugs were dissolved with H2O at a volume of 5 ml/kg body weight. The doses referred to free bases.

Postmortem examination: Following BP determination through the aortic cannula, animals were sacrificed with ether, and inspected macroscopically. The cranial cavity was opened to observe the brain. The heart and kidney were weighed before fixation. Tissues were fixed with 10% formalin, and embedded in paraffin. Hematoxylin-eosin or Elastica-van Gieson stain was performed on the 4 μm sections.

We examined the vascular disease characteristic to hypertensive rats, and determined if death had been caused by the vascular disease. The vascular disease is vascular lesions marked in the kidney, mesenteric arteries, heart, and brain. Intimal thickening and medial necrosis are common microscopical findings. Details of the histopathology will be reported elsewhere.

Diuretic studies

Female rats of HOS®: Donryu strain weighing 250±20 g were placed in metabolism cages. Urine was collected at 30 min intervals for 180 min following bicarbonate saline (NaCl 110 mm, NaHCO3 30 mm) load, 50 ml/kg, p.o. Urine volume (V) was determined at each interval. Osmolality and Cl were measured in the urine samples pooled for 180 min by freezing point depression with an osmometer (Fiske 130), and coulometric titration with a chloride meter (EEL), respectively. Na and K in pooled samples were determined with a flamephotometer (IL 343).

Acute effects of the drugs on BP for 180 min were recorded without anesthesia or restraint through a cannula inserted into the abdominal aorta in normal rats, in order to evaluate the effect of systemic BP on the drug induced urinary changes. The methods of BP determination and drug administration were the same as in the previous section.
RESULTS

Antihypertensive effects of L 6150, hydralazine and ecarazine given orally for 11 weeks

Body weight: In SHR rats, the body weight (BW) increased at approx. an equal rate in all experimental groups, SOL, L4, L12, HZ, and EZ. In DOC and renal hypertension, BW increased to a greater extent in the treated groups than in the control. In fact the drug treatments improved general conditions in these groups. The increase in BW cannot be ascribed to the sodium retaining tendency of the drugs, because the rats showed no sign of retention during 11 weeks. Their sodium metabolism seemed to be accordingly balanced during long term observation.

Blood pressure (Figs. 1–3): In SHR rats given the solvent (SOL) BP continued to increase for 11 weeks. Lower values were obtained by the direct measurement at the 11th week as compared to those at the 10th week, because the tail BP determined indirectly is near the maximum BP while that determined directly through the aortic cannula is the mean BP. In the L4 group, BP fell for 1–11 weeks after initiation of treatment. P values at the 1st, 10th, and 11th week against SOL were <0.02, <0.001, and <0.001, respectively. In L12, HZ, and EZ groups, BP fell more markedly than in L4 for 1–11 weeks after the treatments. P values at the 1st, 10th, and 11th week were <0.001 each in L12; <0.002, <0.001, and <0.001 in HZ; and <0.001 each in EZ, respectively.

In DOC hypertension, BP of the SOL group was usually higher than 180 mmHg during the 11 weeks of observation. In the L4 group BP decreased one week after the treatment and remained at a lower level, but the differences at the 10th or 11th week were statistically not significant against SOL. In L12, HZ, and EZ groups BP decreased for 1–11 weeks after
the treatment. The decreases were larger in an order of EZ, L12, HZ, and L4. P values against SOL at the 10th and 11th week were <0.001 each in L12; <0.02, and <0.001 in HZ, and <0.001 each in EZ, respectively.

In CLIP hypertension, the average values of BP in the SOL group were over 200 mmHg throughout the observation period except at the 3rd and 10th weeks. In the L4 group a transient BP fall was observed 1 week after the treatment. BP rose for 2–3 weeks, then decreased gradually until the 11th week. P values against SOL were <0.001, <0.06, and <0.001 at the 1st, 10th, and 11th week, respectively. In L12, HZ, and EZ groups, BP fell more markedly than in the L4 for 1–11 weeks after the treatments. P values at the 1st, 10th, and 11th week were <0.001, <0.08, and <0.001 in L12; <0.002, <0.02, and <0.001
in HZ, and <0.001, <0.08, and <0.005 in EZ, respectively.

Heart rate: In SHR rats (Fig. 4) HR increased for 1–10 weeks after the treatments with decrease in BP. In DOC hypertension, HR of the L4 was approx. equal values seen in the SOL group for 1–8 weeks, and increased slightly after 9–10 weeks. In L12, HZ, and EZ groups of DOC hypertension HR increased markedly for 1–10 weeks. In CLIP hypertension HR of the L4 was rather lower than that noted in SOL for 2–8 weeks after the treatment. In the L12, HZ, and EZ groups HR did not differ significantly from that noted in SOL for 1–8 weeks, and tended to be higher for 9–10 weeks after the treatments.

Heart and kidney weights: Heart and kidney weights of SHR rats did not differ between drug-treated and untreated groups despite a significant decrease in BP in the former. Heart weights of drug-treated rats were less than those of the untreated (P<0.05 each) in DOC or CLIP hypertension. Kidney weights of L12, HZ, and EZ in DOC rats slightly decreased compared to the SOL, but the differences were statistically not significant. Weights of the clipped and contralateral kidneys in drug-treated rats were also decreased when compared to those of SOL (P<0.05 in L12, HZ, and EZ).

Vascular lesions (Table 2): The vascular disease or tissue injuries caused by the vascular disease was not evident in either SOL or the treated group of SHR rats. In DOC hypertension L 6150 (4 or 12 mg/kg per day) or EZ decreased incidences of the vascular disease and of related death. HZ had no influence on incidence of the vascular disease, but the mortality was diminished. In CLIP hypertension, death due to the vascular disease decreased in the L4 group. Incidence of the vascular disease diminished in L12 and HZ. Related death was not seen in rats treated with L 6150 (12 mg/kg per day), HZ, and EZ.

Microscopically, necrotizing angitis was commonly seen in the vascular disease. In DOC hypertension the lesions were more marked in the arterioles, and resulted in renal

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**Fig. 4.** Effects of L 6150, hydralazine, and ecarazine (orally for 11 weeks) on heart rate in SHR rats. Vertical bars are SE of the means. Number in parentheses following explanation of symbols is the initial No. of animals.
failure, while in the case of CLIP the lesions occurred mostly on the larger arterial wall,
and caused apoplexy.

Renal effects of L 6150, hydralazine and ecarazine (Figs. 5, 6; Table 3)

The doses of L 6150 (12 mg/kg, p.o.), HZ (4 mg/kg, p.o.) and EZ (12 mg/kg, p.o.) were
approx. equipotent in their chronic antihypertensive action. Acute depressor effects of
these drugs observed every 30 min for 180 min after bicarbonate saline load were smallest
with L 6150. The effects of HZ and EZ were equal in degree, and reached a maximum
30 and 120 min after, respectively.

L 6150 increased urine volume (V) 30–60 min after the treatment and bicarbonate saline
load. Urine volume decreased after 60 min, and the total volume for 180 min was also
smaller as compared to control. The pattern of urine output in the L 6150 group was
similar to that in SOL in which a peak output was seen at 30–60 min, and a gradual decrease
was noted at 60–180 min. HZ and EZ decreased urine volume for 180 min to approx. 1/2

FIG. 5. Effects of L 6150, hydralazine, and ecarazine on blood pressure after bicar-
bonate saline load in rats. Vertical bars are SE of the mean. No. of rats is in
parentheses following explanation of symbols. * indicates statistically significant
difference (P<0.05) from BP in control group given the solvent.

| Drug | DOC VD(-) | DOC VD(+) | DOC Death | DOC Total | CLIP VD(-) | CLIP VD(+) | CLIP Death | CLIP Total |
|------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| SOL  | 3         | 7         | 5         | 10        | 0         | 9         | 6         | 9         |
| L4   | 4         | 5         | 2         | 9         | 0         | 7         | 1         | 7         |
| L12  | 5         | 5         | 3         | 10        | 6         | 4         | 0         | 10        |
| HZ   | 3         | 7         | 2         | 10        | 3         | 5         | 0         | 8         |
| EZ   | 5         | 5         | 4         | 10        | 0         | 7         | 0         | 7         |

Figures indicate No. of rats. VD(-) and VD(+) indicate the rats without and with the vascular
disease, respectively. Death refers to that related to the vascular disease. See Table 1 for
the drug treatments.
and 1.3 of that in SOL, respectively. The patterns in HZ and EZ were also different from that of SOL. Peak output was at 150-180 min after the treatment in HZ. The output curve was flat in EZ.

Excretion of osmotically active solutes, Cl, Na, and K decreased with L 6150, HZ, and EZ in parallel with the decrease in V. The decrease in K excretion with L 6150 was relatively larger than those in HZ and EZ when compared with the decrease in urine volume.

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\begin{align*}
&\text{UKV, V ratios were 41±2.5, 26±3.1, 52±5.8, and 84±1.6 (eq ml}^{-1}, \text{mean±SE) in SOL, L 6150, HZ, and EZ, respectively. P value in L 6150 against SOL was }<0.002.
\end{align*}
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**DISCUSSION**

There is no simple and reliable method to determine the antihypertensive effect of a
drug in the preclinical study. Administration of the drug to hypertensive animals for a sufficient duration may be the only possible way at present. Hypertension models in animals are not entirely equal to the types of hypertension in humans. The best model for essential hypertension also has not established. Effects of the antihypertensive drugs were different in animals from those in humans and the effects also varied among the types of experimental hypertensions and different animal species (9). Therefore, we selected three typical experimental forms of hypertension of various origins: SHR, DOC, and CLIP hypertensive rats, in which effect of HZ has been confirmed (9). We extended observations for 11 weeks which corresponds roughly to 10 years of life in humans.

L 6150 (4 and 12 mg/kg per day, p.o.) (L4, L12), hydralazine (4 mg/kg, p.o.) (HZ) and ecarazine (12 mg/kg, p.o.) (EZ) decreased high blood pressure of SHR rats, DOC and CLIP hypertension. The antihypertensive effects were confirmed by the decrease in heart weight in DOC and CLIP. The increase in body weight in these groups may be a sign of improvement in the general conditions by the treatments, because the rats appeared to improve, and there was no sign of sodium retention. Since the antihypertensive effects were determined only for few days in previous reports on L 6150 (1-4), and since no data were available on the antihypertensive effect of EZ (10-11), the present study is the first to confirm the antihypertensive effects of L 6150, and EZ in three types of hypertensive rats for 11 weeks.

HZ and EZ exert their antihypertensive action by acting mainly on the arterial smooth muscle (10, 12). Increase in heart rate and cardiac output with HZ has been attributed to a reflex response to the peripheral vascular dilatation and decrease in BP, although such has been challenged (13). A central nervous system component also has to be considered in the antihypertensive effect of HZ (14). L 6150 increased heart rate by the same mechanism seen with HZ (2-6). The increase was not apparent in CLIP hypertension, and heart rate was decreased in L4 group. At present we have no explanation for this result.

According to the present observations, 12 mg/kg per day, p.o. of L 6150 was approx. equipotent in the antihypertensive action to 4 mg/kg, p.o. of HZ, and to 12 mg/kg, p.o. of EZ. The drug treatments diminished the vascular disease in DOC or CLIP hypertension, as well as incidence of death due to the vascular disease. L 6150 (12 mg/kg, p.o.) was most effective in diminishing the vascular disease in CLIP hypertension.

The diuretic study revealed that L12, HZ, and EZ decreased urine volume, excretion of osmotically active solutes, Cl, Na, and K. The effects were the least with L12, and the most with EZ at the doses used. Since BP decrease was not parallel with the patterns of urine output, time and degree of dilatation of the renal vascular bed should be different among these drugs. There is no report on tubular action of these vasodilator drugs. The results suggest that these drugs have sodium retaining tendency, although there were no signs of sodium retention in the rats presently studied by long term treatments. Potassium-sparing tendency of L 6150 may be favorable when used with potent diuretics.

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