EFFECTS OF PROPRANOLOL ON INFARCT SIZE AND THE IMPAIRED HEMODYNAMICS IN EXPERIMENTAL MYOCARDIAL INFARCTED DOGS

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We studied the effects of propranolol on infarct size and hemodynamic impairment induced by 24 h-coronary ligation. The myocardial infarction produced by the left circumflex coronary artery ligation was more consistent than that induced by the left anterior descending coronary artery ligation, suggesting that the former is a more appropriate experimental model for pharmacological evaluations. Oral treatment with propranolol, 3–30 mg/kg, reduced infarct size and reduced the elevated left ventricular end-diastolic pressure, which was shown to be most closely related with infarct size, in dogs with circumflex coronary artery ligation extending over 24 h. In conclusion, our results indicate that propranolol protects against the enlargement of infarct size and improves the impaired hemodynamics observed in myocardial infarcted dogs with occlusions extending over 24 h, as well as in dogs with less than 24 h-occlusions reported by numerous investigators.

Keywords — myocardial infarction; propranolol; infarct size; hemodynamics

INTRODUCTION

Propranolol, a β -adrenergic blocker, has been shown in experimental studies to limit infarct size following coronary occlusion and occlusion-reperfusion. In these studies, the effects of propranolol were assessed by epicardial electrocardiogram,^{1–3)} the size of myocardial necrosis,^{1–7)} plasma and myocardial creatine phosphokinase (CPK) enzyme analysis,^{3,5,6,8)} hemodynamics,^{3–5,7,8)} and morphological and pathological changes.⁹⁾ On the other hand, Jesmok *et al.*¹⁰⁾ and Peter¹¹⁾ have shown that propranolol does not reduce the size of the infarct. This discrepancy cannot be attributed to the dose of propranolol used or to the different species, because the doses used were almost the same and dogs were usually the species used. There are numerous normal variations and collateral vessels in dog coronary arteries.¹²⁾ Therefore, the ligation of the same site of the coronary artery does not necessarily produce the same degree of myocardial infarction. This variation may account for

the discrepancy concerning the effects of propranolol on experimentally-induced myocardial infarction. Since we found that the ligation of the left circumflex coronary artery (LCX) produced a more consistent myocardial infarction, with regard to the extent of necrosis, as compared with that produced by the ligation of the left descending coronary artery (LAD), we examined the effects of propranolol on not only infarct size but also on hemodynamics in dogs with myocardial infarction extending over 24 h. To our knowledge, only one other study has been carried out using the 24 h-LCX ligation model and propranolol,⁶⁾ resulting in a dearth of information concerning the relationships between hemodynamics and infarct size in the propranolol-treated dogs 24 h after LCX-ligation.

MATERIALS AND METHODS

Surgical Procedure and Experimental Protocol — Adult mongrel dogs of either sex (weighing 8.5–11.5 kg) were anesthetized with sodium

pentobarbital (25 mg/kg *i.v.*). Ventilation was maintained at a rate of 40 cycles/min and a stroke volume of approximately 25 ml/kg was obtained by means of a cuffed endotracheal tube and a Shinano-respirator (SN-480-3). Standard limb leads were attached for observing the change of lead II waves of the electrocardiogram by means of an electrocardiograph (Nihon Kohden MC-12). A left thoracotomy was performed in the fifth intercostal space and the pericardium was opened. The LCX or LAD coronary artery was isolated approximately 1 cm from its origin and ligated, and the incisions were closed. Hyperventilation was maintained for 40–50 min after coronary ligation, to reduce the mortality, and the ventilation rate was gradually decreased to 25 cycles/min for 80–90 min after ligation. Ventilation was removed 150–180 min after coronary ligation and all animals were left to recover for 24 h from anesthesia. Propranolol hydrochloride (Sumitomo) was suspended in 5% gum arabic solution at a concentration of 30 mg/kg and the drug was administered intragastrically immediately after coronary ligation. Twentyfour h after coronary ligation, the dogs were re-anesthetized with sodium pentobarbital (15–25 mg/kg *i.v.*). The heart rate (HR) count was obtained using a heart rate counter (Nihon Kohden AT-600G) driven by the R wave of the electrocardiogram. A left thoracotomy was then performed under conditions of artificial respiration at a rate of 18 cycles/min and a stroke volume of 30 ml/kg. Stroke volume (SV) was measured by a flow probe attached to the aortic arch and the probe was connected to an electromagnetic flowmeter (Nihon Kohden MFV-1200.) A catheter was inserted from the left atrium into the left ventricle to measure the left ventricular pressure (LVP) and the left ventricular end-diastolic pressure (LVEDP). The first derivative of LVP with respect to time divided by LVP ($(dP/dt)/P$) was obtained by an electronic differentiation of the left ventricular pressure pulse (Nihon Kohden EQ-600G). In dogs with unstable hemodynamic parameters due to intense arrhythmia, measurements were

carried out during a short period of relatively stable hemodynamics. Hemodynamic studies were carried out in the LCX-ligated group.

Assessment of Infarct Size — After completing the measurements of hemodynamics, the dogs were exsanguinated and the heart was weighed and sliced transversely into 0.7–0.9 cm sections as shown in Fig. 1. Each of the five slices was incubated with substrate for phosphorylase in 0.1 M sodium acetate buffer, pH 5.6, for approximately 37 °C for 20 min, rinsed in distilled water and placed in Lugol's iodine for 5 min. Following staining, the basal surface of each slice was photographed. The phosphorylase-stained and unstained areas of slice photographs were measured by an MOP-AM03 semiautomatic image analyzer (Kontron Co, Munich, West Germany). Infarct size was expressed as a percentage of the ventricular myocardium, the total unstained areas of five slices $\div$ their total stained and unstained areas $\times 100\%$.

Statistic Analysis — Infarct Size and Hemodynamic Parameters: Analysis of variance (one way) was performed between the control group with LCX-ligation and the control group with LAD-ligation, or between the propranolol-treated groups and the control group for determination of statistical significance. To test for a significant correlation between infarct size and each hemodynamic parameter, the data

FIG. 1. *Hearts Sliced Transversely for Assessment of Infarct Size*

were subjected to the two-variable, least squares linear regression analysis.

RESULTS

Infarct Size

The range of infarct size for each group is shown in Table I. When the infarct size was expressed as a percentage of the intergrated sectioned area of the ventricle, oral propranolol in dose range of 3–30 mg/kg decreased the infarct size significantly, as compared with the control group. Since the dose-response relationships were not so evident, doses of 3–10 mg/kg were adequate for reducing infarct size. Treatment with a dose of 1 mg/kg did not reduce the infarct size in dogs with LCX-ligation significantly. On the other hand, in dogs with LAD-ligation, treat-

ment with propranolol in a dose of 30 mg/kg decreased the mean infarct size, but the difference was not statistically significant.

Moreover, the variance of the extent of necrosis in dogs with LCX-ligation was significantly smaller than in dogs with LAD-ligation in the control group ($p < 0.05$).

Hemodynamic Studies

Effects of Propranolol — As shown in Table II, in dogs with LCX-ligation extending over a period of 24 h, LVP, max. $(dP/dt)/P$ and SV were decreased, and LVEDP was increased in comparison with the findings in the sham operated dogs. HR did not change.

The results of treatment with each of the doses of propranolol employed did not indicate significant effects on these parameters, when

TABLE I. *Effect of Propranolol on Infarct Size*

	Treatment	N	Infarct size
LCX-ligation			
Control		24	33.7 ± 1.5
Propranolol	1 mg/kg	5	31.6 ± 4.7
	3 mg/kg	7	25.6 ± 1.9^a
	10 mg/kg	8	24.1 ± 2.4^b
	30 mg/kg	7	25.4 ± 2.0^b
LAD-ligation			
Control		18	27.6 ± 2.8
Propranolol	30 mg/kg	10	21.1 ± 5.0

N represents the number of dogs. Values for area of necrosis as percentage of total ventricle are presented as mean $\pm$ S.E.M. a) $p < 0.05$, b) $p < 0.01$ versus control.

TABLE II. *Hemodynamic Parameters in Myocardial Infarcted Dogs 24 h after Propranolol Administration*

	N	HR (beats/min)	LVP (mmHg)	max. $(dP/dt)/P$ (s^{-1})	LVEDP (mmHg)	SV (ml/min)
LCX-ligation						
Control	24	161 ± 4	113 ± 4	31.8 ± 1.4	8.3 ± 0.7	4.7 ± 0.2
Propranolol (3, 10 and 30 mg/kg)	22	165 ± 6	110 ± 3	30.6 ± 1.2	6.6 ± 0.4^a	4.8 ± 0.4
Sham operation	10	165 ± 8	148 ± 6	37.8 ± 2.4	1.7 ± 1.1	6.2 ± 0.5

a) $p < 0.05$ versus control. HR: heart rate, LVP: left ventricular pressure, max. $(dP/dt)/P$: maximal value of first derivative of LVP with respect to time divided by LVP, LVEDP: left ventricular end-diastolic pressure, SV: stroke volume.

compared with those of the control group. However, by assembling all the hemodynamic data gathered from dogs treated with the doses of 3, 10 and 30 mg/kg of propranolol, each showed a significant reduction in infarct size and the propranolol treatment appeared to reduce the increase in LVEDP when compared with that of the control group. On the other hand, LVP, max. $(dP/dt)/P$ and SV did not improve by treatment with propranolol (Table II).

The Relationships between Infarct Size and Hemodynamic Parameter — By assembling all the data obtained from the control and propranolol (3, 10 and 30 mg/kg)-treated groups, there were significant relationships between infarct size and LVP ($r = -0.406$, $p < 0.001$; $y = -0.736x + 133.5$), or LVEDP ($r = 0.546$, $p < 0.001$; $y = 0.193x + 1.623$) (Figs. 2 and 3). There was a tendency toward the relationship between infarct size and SV ($r = -0.261$, $p < 0.1$). On the other hand, no relationship was found to exist between infarct size and HR ($r = -0.0957$), or max. $(dP/dt)/P$ ($r = -0.0130$).

DISCUSSION

Numerous studies concerning the effects of propranolol on experimental myocardial infarction

have been carried out. Some of the studies were concerned with the protective effects of propranolol on the experimental myocardial infarction extending up to several hours at most.^{4,5,10} Others have used the short period (40 min—1 h) occlusion followed by reperfusion.^{1,2,7} Furthermore, most studies of experimental myocardial infarction, extending over 24 h, have been carried out on the LAD-ligated experimental model,^{3,8,11} which we have shown gives varying degrees of necrosis. Only one experiment has been carried out using dogs with LCX-ligation extending over 24 h.⁶ The information obtained with the experimental model, which is very sensitive to propranolol does not necessarily indicate the usefulness of propranolol treatment in patients. For this reason, we used the LCX-ligation extending over 24 h as an appropriate experimental model to study the clinical effects of propranolol.

In addition to infarct size, hemodynamics are very important indicators to estimate drug action. Whether treatment with propranolol is of benefit to the impaired hemodynamics 24 h after myocardial infarction occurred has never been studied. However, there are numerous

FIG. 2. Correlation between Infarct Size and LVP
Closed circles represent each dog belonging to the control group. Open circles represent each dog belonging to the propranolol-treated group.
LVP: left ventricular pressure.

FIG. 3. Correlation between Infarct Size and LVEDP
Closed circles represent each dog belonging to the control group. Open circles represent each dog belonging to the propranolol-treated group.
LVEDP: left ventricular end-diastolic pressure.

reports suggesting that the elevation of ST segment in ischemic hearts is attenuated by treatment with propranolol.^{1,3,13)} Our results indicate that treatment with propranolol reduces infarct size and improves the elevated LVEDP, even with a β -blocker more insensitive myocardial infarction model with 24 h-coronary ligation than a β -blocker sensitive model with a combination of occlusion and reperfusion.⁷⁾ LVEDP and LVP were shown to be related to infarct size significantly, especially LVEDP. It is supposed that the elevated LVEDP made the reduced contractility improve in the 24 h-infarcted hearts by compensation based on Frank-Starling relationship,¹⁴⁾ and a stronger compensation was not needed in the propranolol-treated group.

Mortality was reduced by treatment with propranolol, if this agent is administered more than 20–30 min before coronary ligation (data not shown). However, in this study where administration was carried out about 1 min before coronary ligation, the reduction was not so evident because animals very often died from ventricular fibrillation within 20 min after coronary ligation.

Our results strongly suggest that propranolol treatment is useful in the clinical therapy of myocardial infarction.

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